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The Plant Disease Reporter is issued as a service to plant pathologists throughout the United States. It contains reports, summaries, observations, and comments submitted voluntarily by qualified observers. These reports often are in the form of suggestions, queries, and opinions, frequently purely tentative, offered for consideration or discussion rather than as matters of established fact. In accepting and publishing this material the Crops Research Division serves merely as an informational clearing house. It does not assume responsibility for the subject matter.

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ACCEPTANCE OF MANUSCRIPTS

The increase in the volume of pertinent material offered for publication in the Plant Disease Reporter has made it necessary to limit the subject matter and the length of articles accepted. The subject matter should emphasize new things in plant pathology, such as new records of disease occurrence, serious outbreaks and epidemics, conditions affecting development of plant diseases, techniques of investigation including instrumentation, new discoveries in control including new materials and their evaluation. Manuscripts will be limited to 15 double-spaced typed pages. Insofar as possible, material should be presented as graphs rather than tables. Papers cannot be accepted for publication that report routine control experiments, reviews, bibliographies without annotation, results of routine surveys, mere summaries or lists of plant diseases. By following this procedure we hope to continue publishing all articles promptly.

Paul R. Miller

Manuscripts for and correspondence about this publication should be sent to:

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THE PLANT DISEASE REPORTER

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THE RELATION OF RAINFALL AND TEMPERATURE TO LATE BLIGHT OF POTATO AT BURLINGTON, VERMONT

R. A. Hyre[1]

Summary

Twenty years of data on the weather and the occurrence of the potato late blight at Burlington, Vermont, were analyzed by a moving graph method to check further its value for forecasting the disease. The results agreed closely with those of similar studies of Connecticut and Maine data, indicating the usefulness of the method.

INTRODUCTION

A study is continuing of various methods for forecasting late blight of potato and tomato (caused by Phytophthora infestans (Mont.) d By.) in northeastern United States. One phase of this study has been to relate the occurrence of the disease with rainfall and temperature data where reliable disease records are available for a sufficient number of years. Such reliable disease data were recorded by Lutman (5) for the vicinity of Burlington, Vermont, during the 20-year period 1891-1910, inclusive.

METHODS

To forecast blight, the days considered "favorable" for blight are determined. One method used in northeastern United States by the author and others (1, 2, 3, 4) involves "moving" rainfall and temperature graphs. A day is considered favorable for blight if (a) the 10-day total rainfall is about 1.20 inches or more, (b) the minimum temperature is not less than 45° F, and (c) the 5-day average temperature is less than 78°. After a minimum of 10 consecutive blight-favorable days, blight may or may not be forecast. It usually is forecast if the weather is favorable at the end of the 10-day period, and the weather forecast is for continued blight-favorable weather. The disease, then, is expected to occur a week or two later. This method has been quite suceessful for forecasting late blight of potato and tomato (1, 3, 4) and downy mildew of lima bean (2).

The aforementioned method, as well as a modification thereof, was applied to the Vermont data. The modification consisted of a 5-day moving precipitation graph. If the 5-day total was 0.60 inch or more, precipitation was rated favorable for blight. This modification, however, was less useful than the standard method and will not be considered further here.

Lutman's (5) descriptions of blight severity were translated into three categories (none, light, and severe). The category "light" included his descriptions of "little" and "some" foliage blight, as well as 1 year of "a little dry rot" but no mention of foliage blight. The category "severe" included his descriptions of "much" and "severe" blight. When recorded, his date of the first observation of blight is given for the different years. The weather data for June were obtained from the United States Weather Bureau, while data for July, August, and September were transcribed from Lutman's graphs.

RESULTS

Figure 1 shows the analysis of the rainfall and temperature data in relation to the dates of first observation of blight when recorded and to the amount for the season.

In general, the number and/or length of blight-favorable periods increased as the severity of blight increased. For the 4 years when no blight was observed, there was never more than the minimum period of 10 consecutive blight-favorable days at the end of which time blight might or might not have been forecast, the forecast depending on the weather at that time and in prospect. During the 9 years when blight was rated "light" the blight-favorable periods were generally longer or more numerous than during the years when blight was absent. Also, during the 7 years when blight was rated severe, the blight-favorable periods were longer or more numerous than during the years of little blight.

The Vermont data indicate that late blight might have been fairly accurately forecast for

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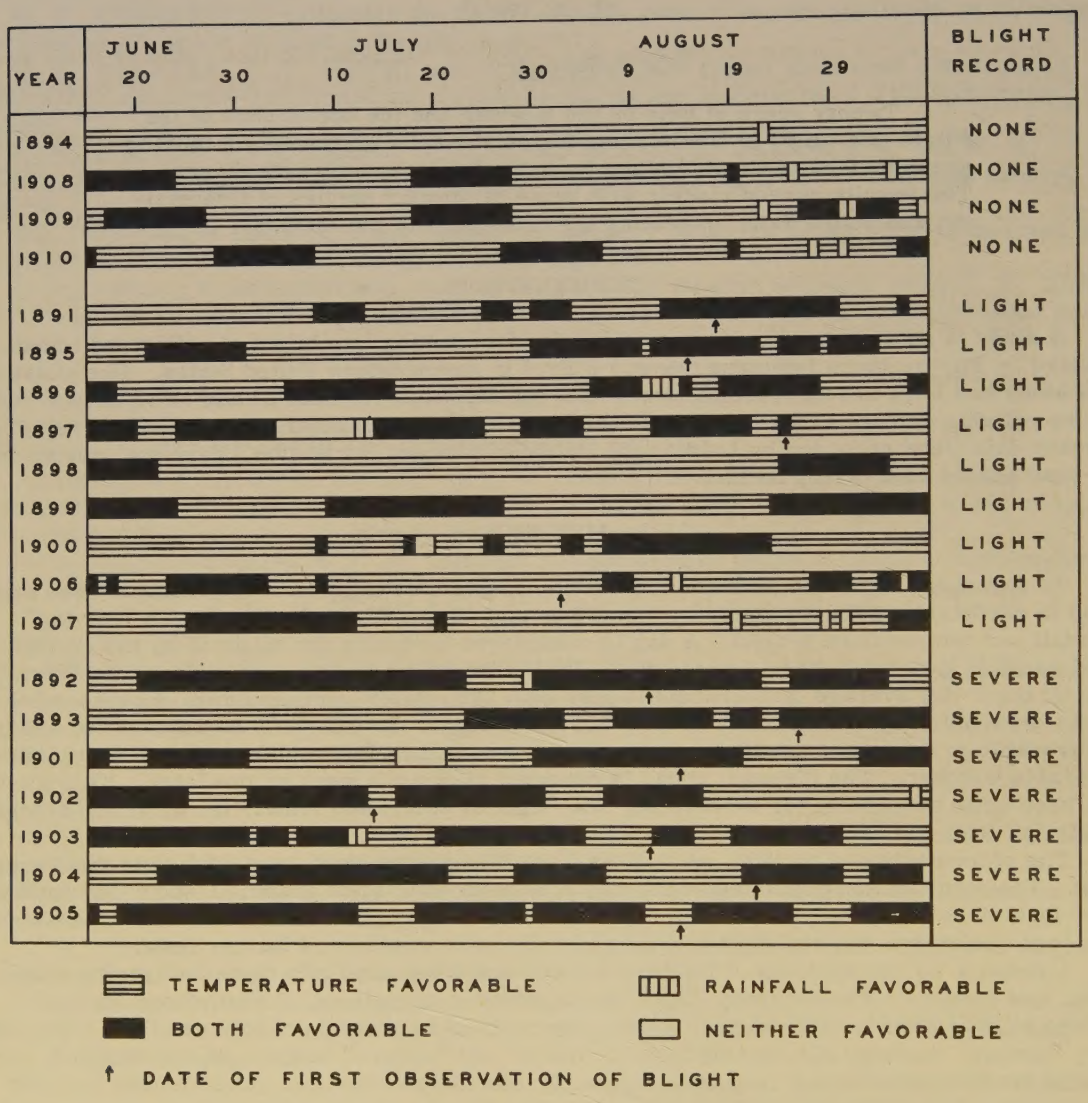

FIGURE 1. A summary of the rainfall and temperature data for Burlington, Vermont in relation to the severity of late blight of potato.

this area from rainfall-temperature data. The errors would have been on the less hazardous side; that is, of forecasting blight when it did not appear, rather than vice versa.

DISCUSSION

There is general agreement between this study for Vermont and those studies made previously for Maine and Connecticut. Thus, it appears that the method used for forecasting blight would be useful for most of northeastern United States.

This study re-emphasizes that the method outlined is not cut and dried but that skill and experience are necessary for best results. Blight does not always follow, and should not necessarily be forecast, after every period of 10 consecutive blight-favorable days. A single heavy rainfall of 1.20 inches or more, in a short time interval, will necessarily result in a period of 10 consecutive favorable days, if the temperature is favorable at the same time, but blight may not be forecast.

The significance of the date of first observation of blight is a moot question, as this date may differ considerably from the actual date of first occurrence.

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EVIDENCE OF EXISTENCE OF PHYSIOLOGIC RACES OF ALTERNARIA SOLANI[1]

Robin G. Henning[2] and L. J. Alexander

Summary

Evidence of the existence of physiologic races of Alternaria solani has been obtained. From 180 single-conidial isolates obtained from infected tomato leaves collected in Ohio, 10, selected on the basis of cultural differences, were tested for pathogenicity. Seven of these appeared to be distinct physiologic races.

A fragmented mycelial suspension of each isolate was sprayed on approximately 6-weeks-old seedlings of Lycopersicon spp. The host seedlings were domestic and wilt species of the genus Lycopersicon, including L. esculentum, L. pimpinellifolium, L. hirsutum var. glabratum, L. peruvianum type spp. and var. humifusum, and L. glandulosum. The host lines were selected on the basis of degree of infection by Alternaria solani in field plots at Wooster, Ohio.

The degree of infection was determined by the average number of lesions per inoculated leaflet. Three types of lesions occurred: small, restricted, necrotic lesions; larger, spreading, water-soaked lesions; and an "intermediate" type, resembling the small, necrotic lesions, but spreading into the leaf veins. Alternaria solani was isolated from all three types of lesions.

The Alternaria solani isolates also differed in their ability to produce lesion development from the point of puncture in a leaflet and in their ability to produce lesions on non-punctured leaflets. Plants were inoculated by placing a single drop of inoculum on each of 50 leaflets on five host plants with a wire loop. The epidermis of 25 of the leaflets was punctured with a steel needle under the drop of inoculum.

The lesion development on the punctured leaflets was evaluated by measuring the diameter of the lesions. The average mean diameter of lesion development per leaflet varied from none to 10 millimeters and each isolate exhibited a different pattern of lesion development on the different host lines.

Three isolates were weakly pathogenic on non-punctured leaflets of all host lines. Each of the remaining nine isolates tested exhibited relatively strong ability to produce infection on leaflets of at least one host line, but each of these isolates exhibited a selectivity of host line.

The patterns of pathogenic reaction of the isolates were not consistent for the experiments described. Discrepancies in correlation of results of the experiments were attributed to non-homogeneity of the host plants, and to variation within cultures of the isolates during the time between experiments.

INTRODUCTION

As a part of the North Central Regional Project NC-7 a large number of accessions of the genus Lycopersicon were grown at the Ohio Agricultural Experiment Station at Wooster, Ohio for the purposes of seed multiplication, evaluation for disease resistance, classification for horticultural characteristics, and species identification. All of these accessions were inoculated in the field with the early blight organism, Alternaria solani, using as inoculum ground dried tomato leaves which had been severely infected or killed by the early blight pathogen. A wide range of severity of infection was observed, and certain accessions first appeared to be

1 Published with approval of the Director of the Ohio Agricultural Experiment Station as Journal Article No. 3-59.

2 Formerly a research assistant at Ohio Agricultural Experiment Station.

resistant but later in the season became affected with early blight. This suggested the possibility that physiologic races of the pathogen existed. Studies on the biology of the causal organism, Alternaria solani, were then undertaken to ascertain whether physiologic races of this fungus do exist.

The importance of the early blight disease to the tomato-growing industry is indicated by the volume of literature that has been published concerning it. The disease has been a serious problem in the southern States in which the bulk of shipped tomatoes and tomato seedlings are produced for the more northern States, and also in the market and canning tomato areas in Ohio and other States where tomatoes are grown extensively. McWhorter (11) in Virginia and Young (19) in Texas included early blight among the important tomato diseases which occur in their respective States.

Other workers have studied Alternaria solani in relation to the early blight disease of tomato and potato: Bonde (1), Brock (2), Groves and Skolko (4), Higgins (6), Locke (9), McCallan and Chan (10), Moore (12), Moore and Thomas (13), Neergaard (14), Nightingale and Ramsey (15), Pound (16), Pound and Stahmann (17), Wellman (18), and others. However, the emphasis in these studies was placed primarily on control, resistance, or conditions favoring the development of the disease. Strains of A. solani have been described on the basis of cultural characteristics or pathogenic virulence by Bonde (1), Brock (2), Higgins (6), Neergaard (14), and Wellman (18). Brock (2) also reported slight differences in pathogenic reaction on the host. Henning and Alexander (5) reported on preliminary experiments, which indicated that physiologic races of A. solani exist. This paper is an elaboration of that work.

If physiologic races of the pathogen Alternaria solani exist and can be demonstrated, progress in developing tomato varieties resistant to the early blight disease would be facilitated. The investigations described in this paper were conducted to determine whether physiologic races of A. solani exist and to provide more information concerning the biology of the pathogen.

MATERIALS AND METHODS

The first Alternaria solani isolates were obtained from fragments of diseased leaf tissue of diseased plants in the plant introduction field plots at Wooster, Ohio. Single conidia were isolated from cultures of the first isolates with a De Fonbrune pneumatic micromanipulator. All other isolates were obtained as single conidia directly from lesions on infected tomato leaves. The lesions were cut out of the leaf, pressed lightly against a hardened drop of 1.2 percent water agar on a cover slip which was then placed inverted on a moist chamber on the stage of a microscope. Individual conidia were then picked off the agar drop with the micromanipulator, using a needle made from 1 mm glass capillary tubing, and transferred to a second, sterile, agar drop on another cover slip. This cover slip was then placed on a glass ring in a Petri dish with moistened filter paper where the spore was permitted to germinate. After 4 to 8 hours each individual conidium was examined and those that had germinated were transferred with a sterile spatula to 1.7 percent potato-dextrose agar in Petri plates and incubated at room temperature (24° to 27° C). After a few days mass mycelial transfers were made to potato-dextrose agar slants that were retained as stock cultures for successive experiments. The isolates used in the pathogenicity tests and their source are listed at the top of the following page.

The isolates differed considerably in cultural characteristics, and many saltated, giving rise to sectors resembling other, non-saltating isolates.

An attempt was made to group the isolates according to cultural characteristics, color of mycelium, pigmentation of the medium, and rate and extent of growth. Although there were striking differences, there was much intergrading between the groups arbitrarily set up.

For pathogenicity tests, 10 of the most strikingly different isolates were selected from the 180 single-conidial isolates that had been obtained. Isolates differed most in color of mycelium and pigmentation of the medium. The majority of the cultures were gray or black with shades between the two. Less frequently, isolates produced white, grayish-orange, red or brown mycelium. Pigmentation of the medium varied from none to a deep wine-red. Conidia of Alternaria tenuis were commonly found on the early blight lesions. Two isolates of A. tenuis were included in the pathogenicity tests.

Source of Isolates

A. solani culture designation	P. I. number or variety	Species	Where obtained
A-2	124162	L. esculentum	Wooster, Ohio
E-1	126953	L. pimpinellifolium	Wooster, Ohio
I-1	129157	L. hirsutum, var. glabratum	Wooster, Ohio
M-1	--	L. esculentum	Massillon, Ohio
M-4	--	L. esculentum	Massillon, Ohio
PI-2-g	Random collections from horticultural varieties of L. esculentum		Marietta, Ohio
PI-3-a	" " "	" " "	Marietta, Ohio
T-1-a	"	" " "	Marietta, Ohio
T-5-a	"	" " "	Marietta, Ohio
T-6-a	"	" " "	Marietta, Ohio
T-10-f	"	" " "	Marietta, Ohio
T-11-b	"	" " "	Marietta, Ohio
T-32-d	"	" " "	Marietta, Ohio
T-41-c	Southland (L. esculentum)		Marietta, Ohio
T-51-b	Resistant breeding line, L. esculentum		Marietta, Ohio

A description of the isolates used in the plant tests are given in Table 1.

Cultures of these isolates were grown on potato-dextrose agar at room temperature (24° to 27° C). Photographs of the cultures at age of 10 days are shown in Figure 1.

Hosts for the pathogenicity tests were selected from the plant introduction accessions on the basis of their varying reaction to Alternaria solani infection in the field plots at Wooster, Ohio. All were species of the genus Lycopersicon, including the two red-fruited species, Lycopersicon esculentum and L. pimpinellifolium, and three green-fruited species, L. hirsutum, L. hirsutum var. glabratum, L. peruvianum type spp., and var. humifusum, and L. glandulosum. Identification and Alternaria reaction of these hosts was reported by Hoover et al. (7) and are shown in Table 2.

Seeds of each host were obtained from mature fruits of plants of the Lycopersicon accessions grown and evaluated in the Plant Introduction field plots at Wooster, Ohio.

The pathogenic reaction of the Alternaria isolates was tested on young potted plants, approximately 6 weeks old. Inoculum of each isolate was prepared by mixing the mycelium of each culture with the agar on which it grew in a Waring blendor for 2 minutes following the method described by Dorrell and Page (3). Sterile distilled water was added during the blending process to dilute the mixture so that it could be sprayed. This inoculum was sprayed onto 10 individual plants of each host line with a DeVilbiss atomizer operating at 25 pounds air pressure per square inch. Care was taken to cover both surfaces of all leaflets. After the plants were sprayed they were placed in a humidity chamber for 48 hours, then removed to greenhouse benches where they were allowed to grow for 8 days, after which notes were taken. The temperature in the humidity chamber was held at approximately 23° C and in the greenhouse at about 16° C. Pound (16) reported these temperatures to be the most favorable for promoting incidence and development of the early blight disease.

Degree of infection was determined on the basis of the average number of lesions per inoculated leaflet. Six classes were established, covering a range of infection from none to severe (Table 3). Five or more lesions per inoculated leaflet was considered to be a severe infection; three, a medium infection; and one, a slight infection.

RESULTS

Results of the pathogenicity tests are shown in Table 4. There were distinct differences in the pathogenic reaction of isolates on the hosts. The most striking was the general pathogenic virulence of the isolates on some or most of the host lines. For example, isolates I-1, T-41-c, and T-10-f were highly pathogenic on some lines, whereas isolates A-2, T-6-a, T-51-b, and

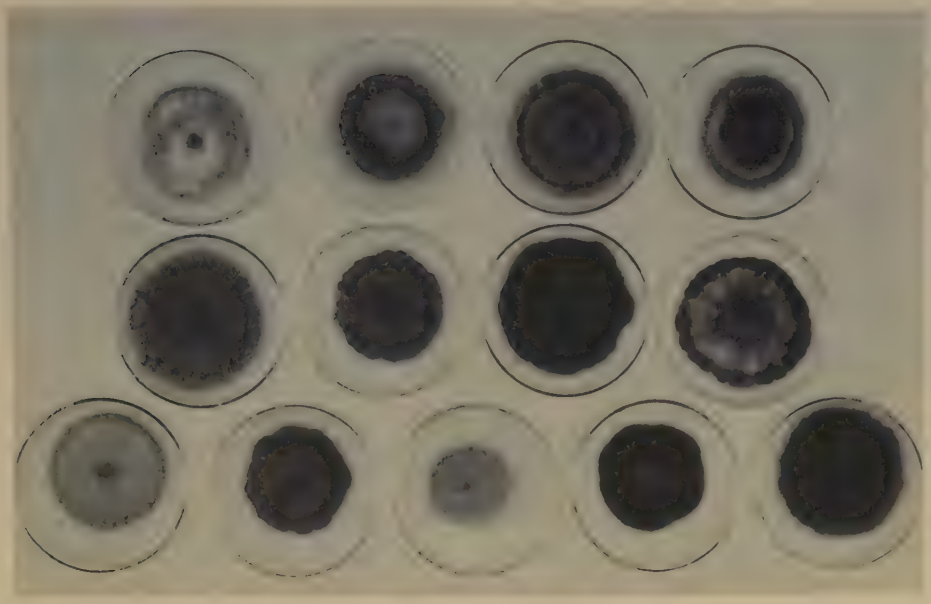

FIGURE 1. Variation exhibited by isolates of Alternaria solani and A. tenuis after 10 days growth on PDA at room temperature. Top row left to right (1) I-1, (2) T-51-b, (3) PI-2-g, (4) A. tenuis isolate; center row (5) T-10-f, (6) A-2, (7) T-1-a, (8) A. tenuis isolate; bottom row (9) T-6-a, (10) T-41-c, (11) T-42-b, showing definitely restricted growth, (12) E-1, (13) M-4.

FIGURE 2. Small type lesions produced on tomato leaflets by Alternaria solani. Left and center, severely infected leaves. Right, healthy leaf.

Table 1. Macroscopic characteristics of Alternaria solani and A. tenuis isolates on potato-dextrose agar.

Isolate	Mycelium color	Medium pigmentation	Growth characteristics
I-1[b]	Light grayish orange	Deep red	Flat, smooth and velvety
A-2[b]	Gray	None	Flat, smooth and dense felted
T-6-a[b]	Light pinkish gray	Deep red	Med. flat, smooth and dense felted
T-41-c[b]	Sooty-gray-brown	Moderate red	Med. flat, smooth, and dense cottony, slow growth
T-51-b[b]	Gray	Moderate red	Med. flat, smooth, loose felted
T-10-f[b]	Dark brownish gray	Moderate red	Med. flat, smooth, dense cottony
PI-2-g[b]	Dark gray	Deep red	Med. flat, ridged, loose felted
M-4[b]	Light gray	None	Med. flat, frayed, dense cottony
E-1[b]	Sooty-gray-black	None	Med. flat, tufted, frayed, cottony, often submerged
T-1-a[b]	Sooty-black	None	Med. flat, frayed, dense cottony
T-8-f[a]	Gray-olive-green	None	High, tufted, billowy, loose cottony
T-43-i[a]	Olive green	Slight browning	Med. high, tufted, dense felted

[a] Alternaria tenuis
[b] Alternaria solani

Table 2. Susceptibility of hosts to Alternaria solani in field plots.

Host	P. I. number	Species	Susceptibility
1	79532	L. pimpinellifolium	Slight-medium
2	126436	L. pimpinellifolium	Medium
3	128645	L. peruvianum	Medium
4	129135	L. peruvianum	Medium-severe
5	126441	L. glandulosum	Slight-medium
6	126443	L. glandulosum	Medium-severe
7	126448	L. glandulosum	Medium
8	127829	L. peruvianum var. humifusum	Very slight
9	126445	L. hirsutum	Very slight
10	129152	L. hirsutum var. glabratum	Slight
11	111407	L. esculentum	Very severe
12	138630	L. esculentum	Slight

PI-2-g were generally only slightly pathogenic on all host lines, and isolate E-1 was almost entirely non-pathogenic. A closer examination of the data revealed that qualitative differences in the pathogenicity of each isolate existed. Certain of the host lines were severely infected by one isolate whereas other lines were slightly infected. For example, isolate I-1 was severely pathogenic on host lines 5, 6, 9, and 10, but slightly pathogenic on lines 1, 2, 4, 8, and 11.

These qualitative differences in pathogenicity varied from isolate to isolate, but each isolate exhibited a distinct qualitative pattern of infection of its own (Table 4).

Isolate I-1 was slightly pathogenic on hosts 1, 2, and 11, but severely pathogenic on host lines 5, 6, 9, and 10, whereas isolate T-41-c was moderately to severely pathogenic on the former hosts, but slightly to moderately pathogenic to the latter hosts. Isolate I-1 was slightly pathogenic to hosts 1, 2 and 11, but severely pathogenic to hosts 3, 5, 7, 9 and 10, whereas isolate T-10-f was severely pathogenic to former hosts but slightly pathogenic to the latter hosts. Isolate T-41-c was moderately pathogenic to hosts 1 and 6, but was moderately to severely pathogenic to host 3, whereas isolate T-10-f was severely pathogenic to the former hosts but was slightly to moderately pathogenic to host 3.

Qualitative differences in pathogenicity also existed between other isolates. Isolate I-1 differed from isolate T-1-a; T-1-a differed from isolate M-4; isolate T-6-a differed from T-51-b; and isolate A-2 appeared to differ from all others. On this basis seven pathogenic races could be established.

Isolate T-1-a was moderately to severely pathogenic to host line 1, but isolate M-4 was slightly pathogenic to this host, whereas isolate T-1-a was slightly pathogenic to host 12, but isolate M-4 was moderately to severely pathogenic to this host.

Isolate T-6-a was non-pathogenic to host line 1, but isolate T-51-b was slightly to moderately pathogenic to this host, whereas isolate T-6-a was slightly to moderately pathogenic to host line 5, but isolate T-51-b was non-pathogenic to this host.

Isolate A-2 was slightly to moderately pathogenic to host lines 8 and 9 and slightly or non-pathogenic to the other host lines, whereas none of the other isolates were more than slightly pathogenic to host line 8, but were at least slightly to moderately pathogenic to one or more of the other host lines (isolates PI-2-g and E-1 being excepted because of their weak or non-pathogenic reaction on all host lines).

When the above-described experiment was repeated qualitative differences in the pathogenicity of Alternaria solani isolates were observed, but they were not consistent for all the isolates with the differences described earlier. Since the seed from which the tomato seedlings were grown was the same in both experiments, and cultures of the isolates with which the host plants were inoculated were transferred out of the same stock cultures, some unknown factor must have influenced the pathogenicity of some of the isolates. Results of the duplicate experiment are given in Table 5.

The discrepancies in the results of the two experiments may probably be attributed to variations in the hosts and the isolates since the seedlings were probably not homozygous. Bonde (1) and Neergaard (14) have reported that isolates of the fungus are known to saltate readily in culture.

Another phenomenon observed in this study of Alternaria solani was a difference in the lesions produced by the pathogen on the host plants. Three types of lesions occurred on inoculated tomato leaflets. Those of the first type (Fig. 2) were small, (1 to 3 mm mean diameter) dark brown and necrotic. Those of the second type of lesions (Fig. 3) were larger, (3 to 5 mm mean diameter) although occasionally some were larger, lighter in color, and enlarged more rapidly. These two types of lesions were distinct and occurred both singly on certain host lines as shown in Figures 2 and 3, and together on plants of the same host, or even on the same plant (Fig. 4). The third type was an "intermediate" type which resembled the small necrotic lesions, but were larger and spread along the leaf veins, sometimes killing the tissue between the veins. This type was not as distinct but was recognizable as being different from either of the first two types. An example of the third type can be seen in Figure 4. The pathogen was reisolated from the tissue of all three types of lesions, but no cultural differences were noted between cultures of these reisolates. No tests have been made to ascertain whether these reisolates exhibit pathogenic differences.

An attempt was made in a third experiment to reduce the number of variable factors which made reproduction of results of this type of experiment difficult. A more objective method of evaluation of infection by Alternaria solani suggested by the work of Locke (8) and Wellman (18) was followed. The inoculum of the isolates was prepared as described above, but the plants were inoculated by placing a single drop of inoculum, approximately 3 mm in diameter, on 10 leaflets of each host plant with a wire loop. Five of the leaflets, half of the number inoculated, were punctured with a needle under the drop of inoculum. Five plants of each host were inoculated, giving a total of 50 individual inoculations for each host; 25 in which the leaflets were punctured, and 25 in which the leaflets were not punctured. The infection of the hosts of A. solani was evaluated by measuring the diameter of the lesion on punctured leaflets and by the number of lesions that developed on the non-punctured leaflets.

Plants were approximately the same age as those used in the previous experiments and were inoculated as previously described. Seven of the more pathogenic isolates of Alternaria

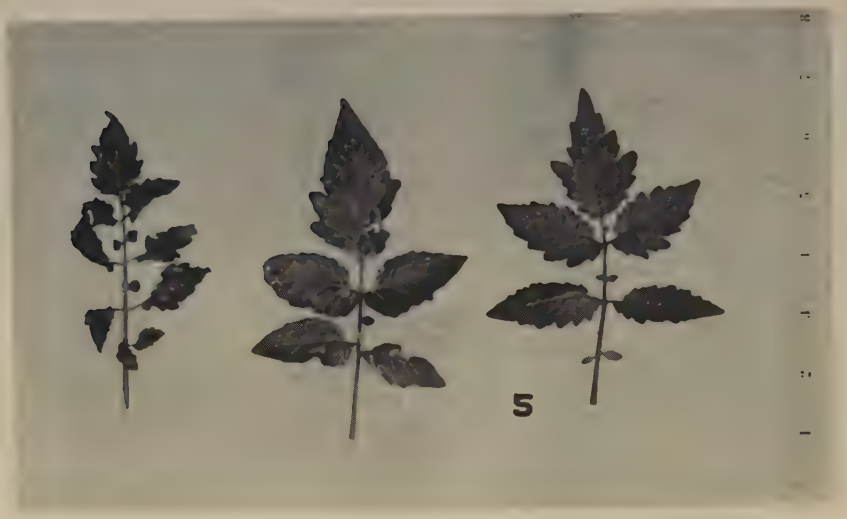

FIGURE 3. Large type lesions produced by Alternaria solani. Left, very severely infected leaf. The lesions have enlarged and coalesced, involving entire leaflets. Center, less severely infected leaf. Right, healthy leaf.

FIGURE 4. Both small and large types of lesions on the same leaves, with some intermediate type lesions, indicated by arrow. Left and center, severely infected leaves. Right, healthy leaf.

Table 3. System used to describe degree of infection.

Average lesions per leaflet	Rating	Description of infection
0	--	None
1	+	Slight
2	++	Slight to medium
3	+++	Medium
4	++++	Medium to severe
5	+++++	Severe

Table 4. Pathogenic reaction of Alternaria solani isolates on Lycopersicon spp.

Host line	Alternaria isolates									
	I-1	A-2	T-6-a	T-41-c	T-51-b	T-10-f	PI-2-g	M-4	E-1	T-1-a
1	+	+	-	+++	++	+++++	+	+	-	++++
2	++	+	-	+++++	+	+++++	-	+	-	+
3	++++	+	-	++++	-	++	-	+	-	+
4	++	+	-	+++	-	++	-	+	-	+
5	+++++	+	++	++	-	+++	+	++	-	+
6	+++++	+	-	+++	+	+++++	-	+++	+	+
7	++++	+	-	+++	-	++	+	+	-	+
8	+	++	-	+	-	+	+	+	-	+
9	+++++	++	-	++	-	++	-	+++	-	-
10	+++++	+	+	++	-	++	-	+++	-	+
11	+	-	-	+++	+	+++++	+	+	-	+
12	+++	-	-	++++	+	+++	+	++++	-	+

Table 5. Pathogenic reaction of Alternaria solani isolates on Lycopersicon spp. Duplicate experiment of results shown in Table 4.

Host line	Alternaria isolates									
	I-1	A-2	T-6-a	T-41-c	T-51-b	T-10-f	PI-2-g	M-4	E-1	T-1-a
1	+	++	+	++++	++++	+	+	-	-	-
2	++	++	+	+++++	+++	++	+	+	-	-
3	+++	+++	-	++++	++	+	-	+	-	-
4	++	++++	+	++++	++	++++	+	+	-	+
5	++++	+	-	++++	+	+++++	+	+	-	-
6	++++	++	-	+++++	+++	+++	-	+	-	+
7	++++	+++	-	++++	+	++	+	+	-	+
8	-	++	+	++	+	+	+	-	-	-
9	+++	+++	-	+++++	+	+++++	++	+	-	+
10	++++	++	-	++++	+++	++++	++	-	-	+
11	++	+++	-	+++++	+++	++++	+	++	-	+
12	++++	++++	+	+++++	++	+++	+	++	+	+

Table 6. Development of lesions on punctured leaflets of Lycopersicon spp. seedlings inoculated with A. solani isolates. Average mean diameter of lesion development in millimeters.

	Isolates											
Hosts	I-1	A-2	T-41-c	T-10-f	PI-2-g	M-4	T-1-a	T-5-a	PI-3-a	T-32-d	M-1	T-11-b
1	1.4	0.5	0.1	0.4	0.1	0.0	1.7	0.8	0.1	2.6	0.9	0.7
2	1.1	0.3	0.1	3.1	0.3	0.0	2.4	1.5	0.0	2.7	1.9	0.8
3	0.3	1.8	0.8	3.4	2.6	0.2	6.3	1.1	2.3	2.6	2.1	3.2
4	0.8	2.1	0.4	5.1	1.4	0.7	6.2	1.2	0.5	3.1	1.9	3.6
5	0.8	1.3	0.2	-	0.3	0.4	4.1	3.1	0.6	3.0	0.6	1.1
6	1.0	3.2	0.2	3.7	0.0	0.2	4.8	2.0	0.5	2.1	0.9	2.9
7	0.4	3.0	0.2	6.8	2.2	0.1	4.9	1.0	0.6	2.1	1.5	2.8
8	0.8	0.8	0.1	2.3	5.2	0.8	7.4	0.2	1.6	1.8	1.4	2.1
9												
10	1.1	4.0	1.1	10.0	4.9	0.2	9.6	2.1	7.1	2.9	8.7	7.5
11	0.3	0.3	0.2	0.0	0.3	0.0	3.4	1.3	0.3	1.6	0.0	1.4
12												
Bonny Best	2.1	2.6	1.0	5.0	0.3	1.4	4.0	3.2	0.7	4.2	0.4	1.5

Table 7. Penetration of host leaflet epidermis by A. solani isolates. Number of leaflets penetrated.

	Isolates											
Hosts	I-1	A-2	T-41-c	T-10-f	PI-2-g	M-4	T-1-a	T-5-a	PI-3-a	T-32-d	M-1	T-11-b
1	0	9	0	2	0	0	0	0	0	1	2	0
2	0	2	0	3	0	0	1	1	0	1	2	0
3	0	8	0	9	0	0	1	0	2	2	0	6
4	1	9	0	13	0	9	12	0	0	10	3	12
5	2	3	0	-	0	2	1	0	0	2	0	0
6	0	15	0	17	0	2	10	3	0	6	0	11
7	0	6	0	19	0	7	13	0	0	8	5	5
8	0	0	0	21	2	4	1	0	0	15	2	1
9												
10	1	0	0	8	0	1	0	1	0	17	4	0
11	0	5	0	0	0	0	1	0	0	0	0	3
12												
Bonny Best	16	10	0	4	0	15	3	8	0	24	0	1

solani used in the earlier experiment plus five others were used in this experiment. The results of this experiment are shown in Tables 6 and 7. The numbers in Table 6 represent the average mean diameter of lesions, in mm, for 25 leaflets, and in Table 7, the number of lesions that developed on the inoculated, non-punctured leaflets.

Reactions of the isolates on the hosts are shown in Table 6. Isolate I-1 produced greater lesion development on hosts 1 and 2 than did isolate A-2, but less than A-2 on hosts 3, 4, 6, 7, and 10. Isolate T-10-f produced greater lesion development than isolate PI-2-g on hosts 2, 4, 6, 7, 10, and the commercial variety Bonny Best, but less than isolate PI-2-g on host 8. Isolate T-5-a produced greater lesion development than isolate PI-3-a on hosts 2, 5, 6, and Bonny Best, but less than isolate PI-3-a on hosts 3, 8, and 10. Many other such differences can be cited between the patterns of reaction of other isolates.

The Alternaria solani isolates also differed in their ability to produce lesions on non-punctured leaflets. This ability did not seem to be closely correlated with the ability of the isolates to produce lesion development on the punctured leaflets. The number of inoculated leaflets

penetrated are shown in Table 7. These were the next older leaflets on the same host plant as those from which the lesion development data in Table 6 were taken.

Isolates I-1 and T-41-c used in this pathogenicity test were markedly different in their cultural appearance than when used in the plant tests described earlier in this paper. The cultures grew less rapidly, the periphery of the cultures growing in the agar was ragged, and the aerial mycelium was much reduced in quantity from that of the cultures of these isolates prepared for the earlier experiments. The cultures of these isolates also appeared to be much less virulent in this plant test than in the previous ones. These changes appeared to be characteristic of the variation of Alternaria solani cultures described by Neergaard (14).

DISCUSSION

Strains of Alternaria solani have been described by Bonde (1), Higgins (6), Neergaard (14), Wellman (18), and others. In all this previous work, differentiation of strains was based upon cultural characteristics or pathogenic virulence. For example, Neergaard (14), reviewed the work of Bonde (1927 and 1929) who identified physiological races of A. solani on the basis of: 1) size of spores, 2) pigment production, 3) sporulation, 4) intensity of mycelial growth, 5) other cultural characters on artificial media, and 6) tendency to saltate. Bonde's identification of these races was confirmed by Pittman (1929) and Klaus (1940).

Heretofore, no attempt has been made to differentiate strains of Alternaria solani on the basis of qualitative differences in pathogenicity, that is, selectivity of host.

The results of the experiments described in this paper indicate that different isolates of Alternaria solani exhibit qualitative differences in pathogenicity on tomato plant hosts, and that physiologic races of this pathogen exist. Examination of the results shown in Table 4 reveals that there are eight possible races of the ten isolates tested; namely, I-1, A-2, T-41-c, T-51-b, T-10-f, PI-2-g, M-4, and T-1-a. Each of these isolates exhibited a pattern of pathogenicity qualitatively distinct from that of the other isolates.

Results of the experiment, shown in Table 5, indicated that there were seven possible physiologic races of the ten isolates tested. These seven isolates were of the same group as the eight indicated to be races by the results of the experiment shown in Table 4. In the earlier experiment isolate T-1-a was so weakly pathogenic on all host lines that it could not be identified as a possible race. Isolates T-6-a and E-1 were so weakly pathogenic in both experiments that neither could be identified as a possible race.

It should be noted that the patterns of infection of the individual isolates were not consistent in the experiments described above. In addition to some difference in virulence of the isolates in the various experiments, there were distinct differences in the patterns of infection of some of the isolates. For example, the reactions of isolate T-10-f in the two experiments, the results of which are shown in Tables 4 and 5, exhibited entirely different patterns. At the other extreme, isolate I-1 exhibited nearly identical patterns of reaction in the two experiments, with only slight differences in virulence being apparent.

As stated before, discrepancies in correlation of the results of the experiments were probably due to variation within the isolates and within the host lines. Cultural variations of Alternaria solani have been described by Bonde (1) and Neergaard (14). It is reasonable to assume that invisible changes in pathogenicity occur in cultures of the fungus, as well as visible changes in cultural characteristics. Visible saltation or sectoring occurred in many of the cultures maintined for use in the experiments described in this paper.

The host seedlings grew from seeds obtained from the tomato Plant Introduction accessions that grew in the field plots at Wooster, Ohio. Since no attempt to increase the homozygosity of these accessions had been made, except by collecting seed only from the "typical" plants within each plot, the seedlings used in these plant tests cannot be assumed to be identical. In view of these facts, the results presented in this paper must be considered only as preliminary and not conclusive evidence of the existence of physiologic races of Alternaria solani.

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OHIO AGRICULTURAL EXPERIMENT STATION, WOOSTER

CONTROL OF TOMATO ROOT KNOT IN ARIZONA WITH FUMIGANTS[1]

Robert B. Marlatt and Ross M. Allen[2]

Abstract

Hot, sandy Yuma Mesa soil was fumigated with ethylene dibromide (EDB), mixed chlorinated C_3 hydrocarbons ("D-D" and "Telone"), mixed EDB and D-D ("Dorlone"), and dibromochloropropane at various rates as solid (broadcast) injections 8 inches deep. Fumigants were evaluated by comparing fruit yields and root galling. Analysis of variance showed that EDB at rates of 7 to 10 gallons per acre gave the best performance over a 2-year period.

Limited commercial plantings of fresh market tomatoes have been grown on Arizona's sandy Yuma Mesa in recent years. These have shown that the winter and spring crop can be profitable if the hazards of frost and root knot can be avoided.

Because root-knot resistant varieties (1) were not available in past years, attempts were made to control the nematode hazard by soil fumigation. To be satisfactory for the Yuma Mesa, a nematocide must be effective in almost pure sand at exceptionally high temperatures and must give protection for 9 months, from August to May. Lear and Thomason (3) reported significant increases in tomato yields in California's Imperial County by solid (broadcast) and row applications of ethylene dibromide (EDB) and row applications of D-D (a mixture of chlorinated C_3 hydrocarbons). Root-knot ratings showed that EDB prevented galls significantly better than did D-D or dibromochloropropane (DBCP).

1956 EXPERIMENT

Materials and Methods

From previous observations it was known that it would be difficult to control nematodes on the Yuma Mesa coarse-textured soil when dealing with a highly susceptible crop through a long growing period. For these reasons higher than average rates of nematocides were used. The site selected for the experiment had contained Ranger alfalfa which was found to have a moderate and rather uniform nematode population. The alfalfa was plowed and had decayed fairly well before fumigation.

Solid injections, at a depth of 8 inches, of each fumigant were applied to plots measuring 21 by 50 feet. These accommodated three 7-foot tomato beds, the center bed being used for obtaining yield and root-knot readings. Plots were replicated four times in a randomized block design and were treated with: EDB, 83 percent, "Dowfume W85" (Dow Chemical Co.) 7.3 and 14.6 gpa; DBCP, 97 percent, "Fumizone" (Dow Chemical Co.), 1.5, 2.1, and 4.4 gpa.

Morse's Special No. 498 tomatoes were raised in a seed bed which had been thoroughly treated with methyl bromide and were transplanted to the field plots 17 days after fumigation. Frost protectors of arrow-weeds, wire and kraft paper, as described by Hart and Zink (2), were constructed in mid-October. Nevertheless, a severe frost on February 14 killed most of the plants.

Fruit was harvested from center beds from December 19 until the severe frost. Marketable fruit was graded to three sizes, 5 X 6, 6 X 7, and 7 X 8, which refer to the number of fruits in each of two layers of a 30-pound lug. Treatments were compared by analysis of variance.

The relative root-knot index of the plants that survived the frost was recorded in March. A 0 to 4 rating system was used, with 4 representing the most severely galled roots. Treatments were again compared by analysis of variance.

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[1] Arizona Agricultural Experiment Station Technical Paper No. 512.

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Valuable advice was generously given by Harold W. Reynolds, Nematologist, Agricultural Research Service, Cotton Research Center, Tempe, Arizona.

Results

Yields: All soil treatments resulted in significantly higher yields of marketable fruit when compared orthogonally with the controls. There were, however, no significant differences among treatments.

Root-knot Index: Both EDB treatments provided significantly better root-knot control than did the DBCP applications. The 4.4 gpa DBCP resulted in significantly less galling than the lower rates of this material.

1958 EXPERIMENT

Materials and Methods

A moderate population of nematodes was obtained for this experiment by planting cantaloups in May with a grain drill in very lightly infested soil. The cantaloups were disked a month before fumigating and they had disintegrated quite thoroughly before the plots were prepared.

A solid injection of each fumigant was applied to approximately 10-by 30-foot plots. Each plot accommodated a 7-foot tomato bed and a guard area of about 3 feet between beds. Plots were replicated eight times in a randomized block design. Treatments included: EDB, 83 percent; "Dowfume W85", 10.3 gpa; a mixture containing 18.7 percent EDB and 75.2 percent D-D, "Dorlone" (Dow Chemical Co.), 10 gpa; a chlorinated C_3 hydrocarbon mixture, "Telone" (Dow Chemical Co.), 33 gpa; D-D (Shell Chemical Corp.), 31 gpa; DBCP, "Nemagon" (Shell Chemical Corp.), 10 gpa; controls remained untreated.

Tomatoes were transplanted as in the previous experiment and the mortality was recorded so that any severe phytotoxicity could be noted. Fruit was harvested from February to May, in the absence of damaging frosts. Marketable fruit was recorded as before and treatments compared by analysis of variance.

Roots were again examined and given a root-knot index of 0 to 5, with 5 representing the most severely galled. The indices were compared by analysis of variance.

Results

Phytotoxicity: There were no significant differences between treatments as to the numbers of plants that died and had to be replaced. Apparently no severe phytotoxicity occurred when transplanting 1 month after fumigation. This was further substantiated by the fact that DBCP did not significantly decrease yields. This result contrasts with results obtained by Lear and Thomason (3) in much heavier soil, transplanting 3 weeks after fumigation.

Yields: A combination of severe virus diseases and an unevenly distributed nematode population probably can be blamed for no significant differences between yields from control or treated plots.

Root-knot Index: Plots treated with EDB, Telone, and D-D had significantly less root knot than did the controls. EDB controlled root galling significantly better than did DBCP or Dorlone and Telone was significantly more effective than Dorlone.

In general, EDB at rates of 7 to 10 gpa gave the best performance in the hot, sandy Yuma Mesa soils.

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ARIZONA AGRICULTURAL EXPERIMENT STATION, TUCSON

IMPRACTICABILITY OF CONTROL OF PLANT PARASITIC NEMATODES WITH IONIZING RADIATIONS[1]

Ronald F. Myers and Victor H. Dropkin[2]

Abstract

Irradiation of 11 species of plant parasitic nematodes indicates that the dose required for complete sterilization is variable, reproduction being completely stopped with doses of 40,000 roentgens in only one species. For six other species, doses above 160,000 roentgens were required to stop reproduction. However, a large reduction in the reproduction of most species can be achieved with a dose of 80,000 roentgens.

The irradiation of nematodes in field soil with a cobalt-60 source is not practical because of the length of time required to irradiate even small areas. Since damage to plant roots occurs at levels of irradiation below those required to disrupt the nematode's life cycle, control of plant parasitic nematodes in and on plant roots by ionizing radiations is not feasible.

The feasibility of using radioactivity to control plant parasitic nematodes in soil and roots was investigated. Great variation has been reported in the amount of radiation required to interrupt reproduction of nematodes. Embryo formation in Trichinella spiralis was inhibited by doses below 1000 roentgens (1). Rhabditis pellio irradiated with 4928 roentgens produced 2.5 offspring per female, whereas unirradiated females had an average of 86.5 young (1). Great reduction was found in the proportion of viable eggs in cysts of Heterodera rostochiensis in the first generation after irradiation with 20,000 roentgens and complete sterilization at doses of 40,000 to 80,000 roentgens (2). A large reduction in numbers of both Ditylenchus destructor and Rhabditis sp. was achieved at 48,000 roentgens, with complete sterilization below 96,000 roentgens (6). Direct killing of T. spiralis occurred at 750,000 roentgens (3).

METHODS

Inoculum consisting of enough infested soil and chopped roots to fill 100-ml plastic vials was irradiated at the Hot Laboratory facilities of the Brookhaven National Laboratory. Various cobalt-60 sources operating at 200,000 to 670,000 roentgens per hour were used. The dosages ranged from 40,000 to 500,000 roentgens, but the inoculum was irradiated at 40,000, 80,000, 160,000 and 320,000 roentgens in most series. After irradiation the inoculum was potted in sterilized soil with an appropriate host plant. Each series consisted of three replications of inoculated controls, inoculated but unirradiated controls, and pots with inoculum irradiated at each of the various dosages.

Nematodes were collected from the pots at various times during a 6-month period by screening, by Baermann funnels, by aeration of roots, or when necessary by soil centrifugation technique, and population counts of the different species were made.

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[1] Cooperative investigations of the Crops Research Division, Agricultural Research Service, United States Department of Agriculture, and Brookhaven National Laboratory under the auspices of the U. S. Atomic Energy Commission.

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RESULTS[3]

Table 1 gives the maximum exposure tried at which reproduction was not stopped and the minimum exposure tried at which reproduction was stopped for 11 species of plant parasitic nematodes. The point of complete sterilization is variable. Populations of nematodes which had received levels of irradiation below those required for complete sterilization increased slowly after remaining relatively constant for 2 to 5 months. Most species tested survived 2 months after irradiation above 300,000 roentgens.

Table 1. Effects of radiation from cobalt-60 on reproduction of plant parasitic nematodes.

Nematode species	Reproduction	
	Not stopped (roentgens)	Stopped (roentgens)
Pratylenchus vulnus Allen and Jensen, 1951	0	40,000
Xiphinema diversicaudatum (Micoletzky, 1927) Thorne, 1939	40,000	80,000
Tylenchorhynchus claytoni Steiner, 1937	60,000	80,000
Ditylenchus myceliophagus Goodey, 1958	60,000	120,000
Criconemoides sp. Taylor, 1936	80,000	160,000
Meloidogyne arenaria thamesi Chitwood, 1952	160,000	180,000
M. incognita acrita Chitwood, 1949	160,000	180,000
Helicotylenchus nannus Steiner, 1945	160,000	320,000
Rotylenchus buxophilus Golden, 1956	160,000	320,000
Scutellonema brachyurum (Stenier, 1938) Andrassy, 1958	160,000	320,000
Trichodorus christiei Allen, 1957	160,000	320,000

DISCUSSION

We consider that 80,000 roentgens would be required to a depth of at least 2 feet in the soil to achieve control in the field similar to that which can be obtained with moderate applications of nematocides. Assuming an irradiation time of 10 seconds, it is found[4] that more than 10^{10} curies of Cobalt-60 would be required to deliver 80,000 R to a point 2 feet deep in the soil. The required source would weigh approximately 60,000 pounds with the source shielding weighing approximately 10^6 pounds. These results may prove to be low by a factor of 1000 since self absorption in the source was not considered and point source calculations were used.

Examination of the hypothesis that irradiation of plant roots offers a possible means of preventing introduction and retarding the spread of plant parasitic nematodes requires a survey of root irradiation data. It has been reported (5) that roots of 22 species of woody and herbaceous plants representing a variety of root types showed harmful effects on exposure to 3500 roentgens or less of x-rays, primary roots being retarded and secondary roots reduced in number and length. After root irradiation, the whole plants remained generally behind unirradiated control plants in development. More than 50 percent of the irradiated roots of broad bean were killed at doses between 600 and 700 roentgens (4). In our own experiments roots of rough lemon were killed at approximately 10,000 roentgens of x-rays. It is doubtful that the amount of radiation

[3] Only generalized nematode radiation data which might be used in control are presented in this paper.

[4] Calculations were made by the Health Physics Department of Brookhaven National Laboratories, Upton, New York.

necessary to sterilize nematodes would leave roots in a living condition.

CONCLUSIONS

Control of plant parasitic nematodes in soil and sterilization of nematodes in or on living plants by irradiation are not feasible according to our data.

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ERADICATION OF ROOT-KNOT NEMATODES FROM GRAPEVINE ROOTINGS BY HOT WATER

Bert Lear[1] and L. A. Lider[2]

Summary

Exposure of grape rootings infected with root-knot nematodes to hot-water treatments resulted in eradication of Meloidogyne incognita acrita and M. javanica javanica in the following treatments: 118° F -- 30 minutes; 120° -- 10 minutes; 125° -- 5 minutes; and 127° -- 3 minutes. No injury was observed from these treatments on 16 commercial varieties and 4 experimental selections of grape rootings.

In the establishment of new vineyards, 1-year-old nursery rootings are planted by many growers in preference to non-rooted cuttings. Many growers produce these rootings themselves in nurseries maintained in or near their vineyards. The same nursery site may be utilized for this purpose for many years with little thought given to the prevention of contamination by nematodes. Consequently, a high percentage of rootings may be infected with nematodes when they are planted in a new vineyard. The practice of preplanting soil fumigation of vineyard sites, especially of those sites previously planted to grapes, has proved beneficial and is often a necessity if economical vineyards are to be established. The use of nematode-infected grape rootings to plant such areas renders the use of fumigants ineffective, as the vineyard will once more become uniformly infested. The combination of an efficient soil treatment and subsequent use of nematode-free planting stock is very desirable. The purpose of these studies was to determine whether a hot-water treatment could be devised to ensure a source of nematode-free grape rootings.

Hot water has been reported as successful for treatment of many plants. Birchfield and van Pelt (1) include a long list of references to such treatments. In California grapes probably were first subjected to hot-water treatments as early as 1910 for control of Phylloxera, a root infesting insect[3]. Treatment recommended exposure for 5 minutes at a temperature of 122° F. The California State Department of Agriculture approved hot-water treatment of Phylloxera-infested vines as an alternative to the quarantining of such vines. These regulations (2, 3) called for treatment at 125° to 130° F for not less than 3 minutes or more than 5 minutes.

MATERIALS AND METHODS

Two series of hot-water treatments were conducted using grapevine rootings infected with root-knot nematodes. The first series contained 2-year-old rootings of the variety Black Rose infected with Meloidogyne incognita acrita subjected to six hot-water treatments and 1 methyl bromide treatment (Table 1). The second series consisted of 32 hot-water treatments (Table 2) in which rootings of the variety Perlette infected with M. javanica javanica were treated. Three additional tests were conducted using 16 commercial varieties and 4 experimental selections of grapevine rootings to determine relative tolerance of varieties to hot-water exposures. The following commercial varieties were treated: Red Malaga, Zinfandel, Mission, French Colombard, Petite Sirah, Barbera, Emperor, Tokay, Perlette, Scarlett, Delight, Ribier, Ruby Cabernet, Black Rose, St. George, and Dogridge.

Constant temperature water baths constructed from commercial milk coolers with a capacity of 120 gallons were used as treatment chambers. At least three replications, each comprising 5 to 25 vines depending upon supply, were treated at each exposure. The entire vine

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[3]Flossfeder, F., and L. O. Bonnet. (Undated Mimeograph) Disinfection against Phylloxera by hot water. Division of Viticulture. University of California.

Table 1. Root-knot gall counts from tomato indicator plants grown in soil infested with root sections from grape rootings (Black Rose) exposed to hot-water and methyl bromide treatments.

Treatment		Number root-knot nematode galls per indicator plant			
Temp. (°F.)	Interval (minutes)	1	2	3	Mean
Check	--	217	115	78	137
118	30	0	0	0	0
120	20	0	0	0	0
122	10	0	0	0	0
125	5	0	0	0	0
127	4	0	0	0	0
130	3	0	0	0	0
Methyl bromide 2 lb./1000 cu. ft.	2 hr.	139	62	35	79

was submerged for the desired interval. Immediately after treatment the vines were plunged into a cooling bath at a temperature of 70° F. For evaluation of efficacy of treatment for nematode control, sections of roots were removed from all treated vines, and chopped into clay pots of soil to which a tomato plant was transplanted. After 4 weeks' growth, the tomato plants were washed free of soil and counts were made of the root-knot nematode galls on the roots. Vine reactions to treatment were checked by planting the treated vines either in the field or in sand beds in the greenhouse. After an adequate interval of growth detailed notes were made on top and root development of each rooting; this was usually 6 to 8 weeks, depending upon seasonal conditions in the greenhouse.

EXPERIMENTAL RESULTS

Root-Knot Nematode Control

Results (Table 1 and 2) show that complete eradication of root-knot nematodes was obtained with the following temperatures and exposure times: 118° F for 30 minutes; 120° for 10 minutes; 122° for 10 minutes; 125° for 5 minutes; 127° for 3 minutes; 130° for 2 minutes; and 135° for 2 minutes. Methyl bromide fumigation at 2 pounds/1000 cubic feet for 2 hours was not effective.

Vine Tolerance

No injury to either tops or roots of treated rootings was observed at temperatures below 130° F. Exposures of 3 to 5 minutes at 130° resulted in death of 25 to 30 percent of roots, but vigorous new root growth resulted. Exposures of 2 to 4 minutes at 135° resulted in about 50 percent of roots killed but new roots were produced on the trunk. At 140° for 2 minutes all of the old roots were killed, but new roots were produced on the trunk. At 145° most of the vines were killed. In one experiment some varieties had terminal shoot growth of 2 inches or more at time of treatment. In all treatments this new growth was killed. Subsequent growth from additional buds, dormant at the time of treatment, however, was normal.

DISCUSSION

The application of the hot-water treatments described should benefit the grape grower by ensuring a source of nematode-free planting stock. At the temperatures listed, exposures

Table 2. Root-knot gall counts from tomato indicator plants grown in soil infested with root sections from grape rootings (Perlette) exposed to hot-water treatments.

Treatment		Number root-knot nematode galls per indicator plant			
Temp. (°F.)	Interval (minutes)	1	2	3	Mean
Check	--	208	135	--[a]	171
118	40	0	0	0	0
	30	0	0	0	0
	20	20	0	0	7
	15	67	13	8	29
	10	72	31	50	51
120	25	0	0	0	0
	20	0	0	0	0
	15	0	0	0	0
	10	0	0	0	0
122	15	0	0	0	0
	12	0	0	0	0
	10	0	0	0	0
	8	0	3	0	1
	6	2	19	0	7
	4	24	5	0	10
125	7	0	0	0	0
	6	0	0	0	0
	5	0	0	0	0
	4	0	0	14	5
	3	0	11	0	3
127	6	0	0	0	0
	5	0	0	0	0
	4	0	0	0	0
	3	0	0	0	0
130	5	0	0	0	0
	4	0	0	0	0
	3	0	0	0	0
	2	0	0	0	0
135	4	0	0	0	0
	3	0	0	0	0
	2	0	0	0	0
140	3	0	0	0	0

[a] Indicator plant killed by nematodes.

longer than necessary for nematode control did not result in vine injury. This provides a margin of safety, at least for all varieties tested, to make these treatments commercially feasible. Treatment of vines when dormant, of course, will reduce the hazard of the treatments. Construction of water baths using either steam or electricity as a heat source is relatively simple. Care must be taken to provide a water bath of a size adequate to accommodate the number of vines to be treated. Adequate ratio of volume of water to number of vines treated is important so that the temperature of the water remains constant, especially when the rootings are first submerged.

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SELECTION FOR RESISTANCE TO ROOT KNOT OF WHITE AND RED CLOVER[1]

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Abstract

A number of introductions, lines, and varieties of red (Trifolium pratense), and white (T. repens) clover were tested for reaction to the root-knot nematodes Meloidogyne incognita (Kofoid & White) Chitwood, and M. incognita var. acrita Chitwood. About 400 seedlings which had only a trace or slight galling were selected from among several thousand plants. A number of these seedlings were sibbed or open-pollinated, or both. Inoculation tests of first and second generation progeny indicated the presence of factors for a good field-type resistance.

The susceptibility of white (Trifolium repens) and red (T. pratense) clover to species of Meloidogyne, the root-knot nematodes, undoubtedly plays an important role in the inability of these crops to survive summers successfully in certain areas of the South. That these plants are quite susceptible to root-knot nemas has been pointed out by Allison (1), Bain (2), and McGlohon and Baxter (3). Since the present methods of chemical control are not practical in this instance, resistance in these crops would be desirable. In 1958, the writer (2) suggested the possibility of selecting red and white clover resistant to certain root-knot nemas, and somewhat later in the year McGlohon and Baxter (3) reported "partial" resistance to M. incognita var. acrita in certain clones of white clover. This paper reports results obtained thus far of an intensive search for resistance to M. incognita and M. incognita var. acrita in the aforementioned crops.

METHODS

Pure cultures of the nematodes were maintained on Rutgers tomatoes in 8-inch pots. At the time of inoculation roots of diseased tomatoes were finely chopped and thoroughly mixed with the soil in which they were growing. This mixture constituted the inoculum. Four-inch clay pots were half-filled with the steamed soil (2:1 sand and clay loam mixture) and about 60 ml of inoculum was spread evenly over the surface. The inoculum was covered with about 0.5 inches steamed soil and between 25 and 100 seed were planted thereon and covered lightly with steamed soil. Moisture and temperature were maintained at a level suitable for root-knot development.

Readings were made with the aid of a Magna-Focuser 30 to 60 days after planting and each plant was indexed as to presence of root knot. Severity was based on relative number, size, and distribution of galls. Plants in the none, trace, and slight classes were repotted and grown for further observations. Several thousand seedlings of over 150 introductions, lines, and varieties of red and white clovers were thus tested two or more times. Between 300 and 400 seedlings were selected out of these because their reaction suggested the possibility that resistance was involved.

RESULTS

The term "resistant", as used in this paper, does not imply total absence of galls, nor does it concern presence or absence of egg masses, but rather means that the plant apparently has a root system healthy enough to maintain itself during periods of stress -- such as normal drought of summer. Thus, resistant plants may fit into the none, trace, or slight galling classes. On the other hand, susceptible plants (or ones with moderate to severe galling) would be considered unable to maintain themselves in periods of stress.

None of the lots tested were immune to either species of nematode; however, two white clovers from Sweden and two from The Netherlands had a significantly low index in M. incognita var. acrita, as did another from Sweden in M. incognita. One and 2 F. C. accessions, respectively, of red clover were less susceptible to M. incognita and M. incognita var. acrita than others. Commercial varieties of both red and white (including Ladino) were considered to be

[1] Journal Article No. 773 of the Mississippi Agricultural Experiment Station, State College, Mississippi.

susceptible. Both red and white clover appeared to be more susceptible to M. incognita -- as evidenced by higher indices and fewer surviving selections. All plants in each group were not classed the same but varied from a trace through to severe galling, although in most cases the majority varied from moderate to severe. Reaction of entries to the two nemas was not of the same order -- that is, a group tolerant to one was susceptible to the other.

White Clover

About 50 and 100 seedlings were selected as having possible resistance to M. incognita and M. incognita var. acrita, respectively. These were potted individually in 3-inch pots and later repotted to 6- or 8-inch pots. Within 5 months all but 32 acrita (Figure 1) and 30 incognita selections had been all but killed by root knot. The remaining plants were either selfed, sibbed, or open-pollinated. After about 12 months roots of these plants were examined and only 11 acrita and 10 incognita selections remained significantly free enough of root knot to be considered resistant. The remainder had moderate to severe galling on the original roots but roots of runners remained free enough of galls to maintain the plants in a vigorous condition with ample moisture.

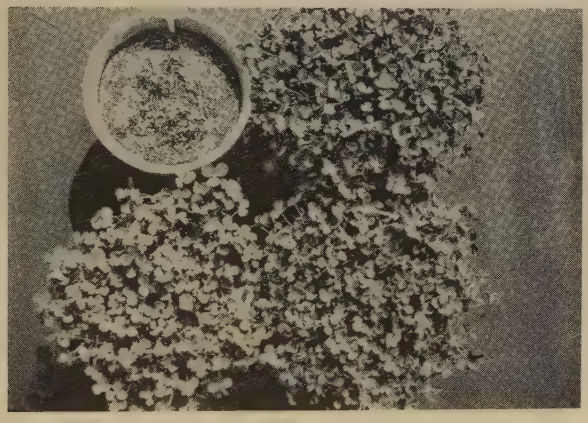

FIGURE 1. Acrita selections of white clover about 5 months after inoculation. The three healthy plants are 27-1, 27-2, 27-11.

Inoculation tests were made with first generation progeny of both acrita and incognita selections. The progeny of three sibbed acrita selections (Figure 1) from a Swedish line (P.I. 195484) in two tests each averaged an index of 3.4 (Table 1), as compared with that of 41.7 for a commercial check variety (Louisiana S-1). In these and other tests the major portions of progeny of other acrita selections which were open-pollinated were classed in the groups falling in the resistant category. Note how data of 27-2 sib and 27-2 op compare. The majority of check plants, on the other hand, were classed in the groups of the susceptible category. The 83 selections from these progeny were examined again about 5 months after repotting and only 16.1 percent had developed moderate or severe galling during that period. It should be noted here that most check plants in the trace and slight classes invariably became moderately to severely galled when repotted and carried over 2 to 3 months.

The indices obtained in one test of the progeny of two sibbed incognita selections from Sweden (P.I. 195485) were likewise low (Table 1). None of the 11 plants selected from 28-3 had developed root knot within 5 months after selection. Results of two tests with seed of 28-3 op also show a major portion of the seedlings were in the resistant category.

Data from a limited number of cross-inoculation tests of acrita selections indicate there is a possibility of selecting for resistance to both nematodes in the lines tested.

An attempt was made to self a number of first generation selections during the late fall in the greenhouse. On two or three occasions, however, a few honey bees got in and worked the clover rather industriously. Consequently, most of the seed of these plants should be considered as coming from open-pollinated (with plants selected for resistance) flowers rather than from selfs. Seed from three of these selections were tested one time for reaction to M. incognita var. acrita. Results of this test (Table 2) show that resistance is present in the second generation in these lines. The female parents of these seedlings were selected from 27-11, the re-

Table 1. Classification and root-knot index of first generation selections of white clover inoculated with M. incognita and M. incognita var. acrita. Notes taken about 40 days after planting. Totals of two tests.

Number	none	trace	slight	moderate	severe	total	index[a]
			Acrita				
27-1[b]	79	17	7	1	0	104	3.8
27-2[b]	72	31	1	0	0	104	3.2
27-11[b]	135	33	10	1	0	179	3.5
check	7	22	56	72	40	197	41.7
19-3[c]	17	42	14	4	13	90	21.6
19-7[c]	19	19	8	2	3	51	14.0
21-2[c]	12	58	40	14	12	136	23.3
27-2[c]	6	26	14	5	5	56	22.0
28-1[c]	0	14	6	0	0	20	14.5
check	0	9	26	20	40	95	49.8
			Incognita				
28-3[b,d]	10	2	0	0	0	12	1.6
28-2[b,d]	2	1	0	0	0	3	3.3
check	0	0	0	1	18	19	73.6
28-3[c]	2	24	14	10	16	66	34.6
check	0	7	20	29	56	112	55.5

a None - 0; trace - 10; slight - 25; moderate - 50; severe - 75.
b Sibbed.
c Open-pollinated.
d One test only.

action of which is given in Table 1. It is not known why there was not more segregation in these progeny unless it was due to open-pollination with other selections.

The facts that progeny of both sibbed and open-pollinated acrita and incognita selections were classed mainly in the resistant category, and that progeny of first generation acrita selections had a low index, strongly suggest the presence of factors for resistance in these lines of white clover.

Red Clover

In general, red clover appeared to be more susceptible to the two nemas than did white clover. This was evidenced by considerably more killing at later stages in growth of plants that had been selected in the seedling stage. Only 14 plants out of over 200 selections survived to produce seed. Seven of the 14 survivors were re-examined after 8 months or more in pots and three had only a trace to slight galling -- the other four (including the only incognita selection) were moderately to severely galled but had survived.

Progeny of sibbed acrita selections were cross-inoculated with both nemas, and those of one open-pollinated selection were inoculated with M. incognita var. acrita only. Results of

Table 2. Classification and root-knot index of second generation selections of white clover inoculated with M. incognita var. acrita. One test, plants open-pollinated. Notes taken 35 days after planting.

Number	none	trace	slight	moderate	severe	total	index[a]
27-11(2)	0	24	5	1	6	36	24.0
27-11(15)	0	38	4	3	0	45	14.0
27-11(18)	0	20	3	0	0	23	11.9
check[b]	0	6	21	13	36	76	51.7

a None - 0; trace - 10; slight - 25; moderate - 50; severe - 75.
b Total of four pots of checks.

Table 3. Classification and root-knot index of first generation selections of red clover inoculated with M. incognita and M. incognita var. acrita. Notes taken 30-40 days after planting.

Number	none	trace	slight	moderate	severe	total	index[a]
			Acrita				
19-3x19-7[b]	16	16	8	1	9	50	21.7
19-7x19-3[b]	3	8	9	14	14	48	42.8
24-2op[c]	6	31	25	12	6	80	24.8
check[b]	0	11	11	51	56	129	55.3
			Incognita				
19-3x19-7[d]	0	13	7	5	2	27	26.1
19-7x19-3[c]	0	1	12	16	8	37	46.2
check[b]	0	6	9	27	73	115	61.8

a None - 0; trace - 10; slight - 25; moderate - 50; severe - 75.
b Total of three tests.
c Total of two tests.
d One test.

these tests (Table 3) indicate the possibility of factors for resistance to both M. incognita and its variety. The distribution of seedlings of the sib 19-3x19-7 (F. C. 24094) and the open-pollinated 24-2 (F. C. 24013) is predominantly in the resistant category (none through slight galling), while the check seedlings were grouped mainly in the susceptible category (Figure 2). It is of interest to note that when 19-7 was used as the female parent in the sib 19-7x19-3 the index, and thus the distribution of seedlings, was in the susceptible category. These results suggest the possibility of cytoplasmic influence on the inheritance of resistance in this particular case. Roots of the parent plants were re-examined about 10 months after selection and 19-3 had only a trace of root knot but the roots of 19-7 were moderately galled. Progeny selected from the sibs were backcrossed to 19-3 and 19-7 but not enough seed were obtained to justify testing.

FIGURE 2. Red clover seedlings about 35 days after inoculation with M. incognita. Check plants are Miss. Kenland; 19-3 are first generation progeny from sibbed (19-3x19-7) acrita selections.

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CONTROL OF PYTHIUM ROOT ROT BY THE NEMATODE APHELENCHUS AVENAE

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Abstract

In pot culture tests in the greenhouse using an autoclaved soil mixture, corn developed very severe root rot when Pythium arrhenomanes was added, remained unaffected when 125,000 Aphelenchus avenae were added per 6-inch pot, and developed only mild root rot when similar numbers of this nematode were added along with the pythium. The nematode fed destructively on the pythium in the laboratory. In the greenhouse it invaded pythium lesions in corn roots but not the healthy roots where pythium was absent. These findings support the view that the population of A. avenae tested is not a root parasite but is instead a beneficial mycophagous nematode that may aid in control of fungus diseases of roots.

The nematode Aphelenchus avenae Bastian is of extremely widespread occurrence in soil and in dead or dying plant parts, and especially in those parts that have been in or on the soil, yet its significance in relationship to plant disease remains uncertain. It thrives in fungus colonies in the laboratory, making it appear that its association with rotting plant parts results from its mycophagous habit. The mycophagous habit, however, does not rule out possible parasitism of higher plants: Aphelenchoides besseyi Christie and Ditylenchus destructor Thorne are both important plant pathogens that thrive in fungus culture. Some nematologists think that, in addition to fungi, A. avenae is able to feed on cells of higher plants, especially in tissues that are in early stages of necrosis (4, 16). Goodey (5) says: "It is often found in situations where it is apparently functioning as a true parasite and it seems clear that it can penetrate and live in apparently healthy plant tissues and function as a facultative parasite." This statement is supported by such reports as that of Steiner (16).

Results of experiments with cotton, reported by Arndt and Christie (1), showed that A. avenae by itself had little effect on seedlings. However, when this nematode was added to soil together with Fusarium moniliforme, before planting the seed, mean top weights of the plants after 2 months were 38 percent less than with the fungus alone. These workers ascribed the difference chiefly to an increased number of stunted plants where the nematodes and the fungus were both present.

Plant tissues may be invaded by fungi before they show distinct symptoms of disease, and fungi may be readily overlooked with the more common methods of nematological examination. Moreover, the population of A. avenae investigated by one of us in Hawaii (12) failed to congregate around healthy roots under conditions that led to conspicuous congregation of three nematode species known to be plant parasites. These facts justify the belief that some of the A. avenae reported within apparently normal plant tissues may have been actually feeding on fungi, and that under some conditions this nematode may be beneficial. Schindler and Stewart (15) reported that a mycophagous Ditylenchus sp. reduced the severity of fusarium wilt of carnation.

During an attempt to diagnose severe root disease in field corn, destructive root rot developed in pot cultures in soil from Will County, Illinois. Pythium arrhenomanes Drechsler was isolated from those roots. When added to steamed soil this fungus caused severe root rot, reproducing the symptoms described by Johann et al. (8). After it was found that A. avenae fed freely on this fungus in the laboratory it became evident that materials were at hand to determine the relationship of this nematode to a root rot caused by a fungus.

MATERIALS AND METHODS

The population of Aphelenchus avenae used in this work was one that had been maintained several years in this laboratory in agar cultures of the fungus Pyrenochaeta sp. Cultures were

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handled essentially as described by Hechler (6). In a 90 mm Petri plate of this fungus on potato-dextrose agar, it is possible to rear over 50,000 individuals during 2 weeks. In such cultures the fungus rarely sporulates. Nematodes were removed from the cultures by a modified Baermann extraction. Decanting and a second passage of the nematodes through a pad of 4-ply facial tissues served to free them from essentially all metabolic products from the old culture and also from the fungus on which they were reared.

The Pyrenochaeta sp. was isolated in this laboratory from roots of spelt and had not been tested for pathogenicity to corn. It was found to be antagonistic to the pythium when the two fungi were planted 4 cm apart on potato-dextrose agar. For addition to pots of soil, the pyrenochaeta was grown 3 weeks on plates of potato-dextrose agar, then suitable pieces of the colony were cut and lifted from the agar with sterilized scalpel and forceps.

The Pythium arrhenomanes was grown on carrot agar (7) for identification and the species was determined on the basis of origin and numbers of antheridia. In preparation for pot culture tests this fungus was grown in potato broth, approximately 15 ml per Petri dish. From a small piece of culture on agar placed in the center of the dish, mycelium grew to cover the entire surface during 48 hours in the dark at room temperature. Sterilized scissors were used at this time to remove the piece of agar from the center and to divide the colony of mycelium in half. Each half was picked up with sterilized forceps, rinsed by dipping in distilled water, and used to infest one 6-inch pot of soil.

FEEDING ON PYTHIUM IN CULTURE

The feeding of A. avenae on pythium is essentially as described for septate mycelia (2, 11). The nematode presses its head against a hypha and immediately begins a rapid thrusting of its stylet. Once the stylet tip is inserted through the hyphal wall it is held well protruded while the median bulb pulsates rapidly. Protoplasm flows to the stylet from both directions in the coenocytic hypha during this pulsation. When pulsation stops the nematode retracts its stylet and moves its head from the feeding site. At this time, protoplasm may gush from the wound in considerable quantities. This has not been observed with septate hyphae of other fungi. Feeding periods are brief but frequent, and a nematode often feeds several times on the same hypha.

To observe effects of the nematode on the growth of P. arrhenomanes, Petri plates of 2 percent water agar were inoculated with uniform pieces of corn meal agar containing the fungus. To some of these plates small numbers of nematodes were added by transferring a piece of agar containing nematodes, but no fungus, from near the margin of a fungus colony on which they had developed.

As the pythium grew sparsely into the water agar, individual hyphae that were seen to be fed on by the nematodes were marked with ink on the bottom of the dish. It was frequently observed that the growth of hyphae stopped when nematodes fed several times at or near the tip. After 2 weeks the quantity of mycelium on plates initially infested with 25 to 50 nematodes was extremely sparse as compared with plates free from nematodes, and many of the remaining hyphae appeared to be devoid of protoplasm. It was apparent that the nematode, by its feeding, had inflicted severe damage to the fungus. Meanwhile, A. avenae had multiplied rapidly.

POT CULTURE TESTS

To determine the effect that Aphelenchus avenae might have on the development of pythium root rot of corn, pot culture tests were made in the greenhouse during May and June 1958. The first test compared two replicates of three treatments. This was followed by an experiment involving five replicates of six treatments.

An autoclaved potting mixture consisting of four parts fine silt loam, two parts rotted manure, and one part sand was placed in 6-inch pots in such quantity that after heavy leaching with tap water the soil level was 1 1/2 inches below the pot rim. Additions of fungi and nematodes, as called for in any treatment, were made in a hole about 1 inch deep in the center of the pot. When pythium was added it was placed in the hole first. Nematodes or pyrenochaeta suspended in 25 ml water were poured on the pythium before the hole was filled. Two kernels of the double cross hybrid (Hy2 X Oh7) (WF9 X 38-11) seed corn were placed directly over this point, then soil was added to fill the pot to 1/2 inch from the rim. After seedlings emerged they were thinned to one plant per pot.

In the preliminary test, two pots received pythium alone, two received pythium plus 125,000 A. avenae, and two were left uninfested as controls. Emergence of seedlings was uniform and growth appeared normal until after 15 days, when plants in the pots with pythium alone began to lag behind the others in rate of growth. These plants soon assumed an abnormally dark green color. The lower leaves gradually turned yellow and blighted, dying back from the tips. The green upper leaves rolled and drooped during the heat of bright days. In pots that received nematodes in addition to the pythium, the plants appeared healthy throughout the test period.

After 28 days the roots were washed and examined. Pythium arrhenomanes, without nematodes, had caused severe brown root rot that involved nearly all of the roots, greatly reducing the functional root system. As expected, roots of the non-infested checks were extensive, white, and free from lesions. Those from pots that received both pythium and nematodes were nearly as extensive as the checks and showed much less root rot than with pythium alone. They did, however, show some areas of severe browning and most of the roots showed small brown lesions. These lesions suggested that the fungus had entered the roots at many points but that the nematode had prevented its extensive development.

FIGURE 1. Part of a root rot lesion from a pot infested with Pythium arrhenomanes and Aphelenchus avenae, after processing in lactophenol with acid fuchsin, showing two nematodes and part of a third, nematode eggs (chiefly at left), and zoosporangia (most evident left of center).

Small representative samples of the several root systems were stained and cleared with acid fuchsin in lactophenol. Extensively browned roots from the pythium treatment contained high concentrations of lobulate zoosporangia in the cortex, usually near the surface and often in root hairs, as described by Johann et al. (8). Severely rotted areas in roots from the pythium plus nematodes treatment also contained many sporangia, but they also contained many nematodes and their eggs (Figure 1). The two root systems of this treatment were then incubated in moist chambers (17) to extract nematodes. During 18 days, the numbers of A. avenae recovered were 209,746 and 206,876, respectively. Most of these were very small larvae that emerged in greatest numbers near the end of this incubation period, and emergence was still continuing when the extraction was terminated. These results indicate that reproduction was continuing in the moist chambers. The incubation time of 18 days is sufficient for the completion of three generations under favorable conditions (unpublished data of H. Carol Hechler in this laboratory).

Results of this preliminary test were so striking that a more adequate experiment was conducted with five replicates of the following six treatments: 1) control; 2) pythium alone; 3) pythium plus 50,000 A. avenae; 4) pythium plus 125,000 A. avenae; 5) pythium plus pyrenochaeta; and 6) 125,000 A. avenae alone. Soon after seedlings emerged a solution containing 0.38 gram KNO_3 was applied to each pot. As in the earlier test, a uniform stand was obtained and early growth was uniform. Then reduced top growth, yellowing and blighting of lower

FIGURE 2. Four-weeks-old corn plants selected to match the mean heights of their respective treatments. Left, control; center, Pythium arrhenomanes alone; right, P. arrhenomanes plus 125,000 Aphelenchus avenae per pot.

leaves and rolling of upper leaves developed in all plants exposed to pythium alone and to pythium plus pyrenochaeta. When the plants were harvested after 28 days, those with nematodes alone or with pythium plus nematodes appeared no different from the controls. Figure 2 shows representative plants of three treatments.

At the age of 4 weeks all plants were cut at the soil surface and the tops were dried in the oven and weighed. Root systems were washed free from soil for examination and small samples were removed for staining and clearing. Root systems were then individually wrapped in squares of muslin, dipped in water, and spun 3 minutes at approximately 410 revolutions per minute in an automatic clothes' washer set in the spin-dry cycle to remove surface water. The muslin was then removed and the roots weighed. The weight data, presented in Tables 1 and 2, were analyzed and Duncan's multiple range test (3) for significance was applied.

Results of this experiment confirmed and extended those of the preliminary test. Pythium arrhenomanes by itself caused severe root rot that severly stunted top growth, and the addition of pyrenochaeta did not significantly reduce disease severity. A. avenae alone had little if any effect on root weight and none on top weight, but similar numbers of nematodes added to pots with pythium served in some way to limit the development of root rot. The data suggest that 50,000 nematodes per pot may have been slightly less effective in suppressing root rot than the larger number, 125,000 per pot, but even the smaller number gave a striking improvement in both root and top weight. As in the preliminary test, nematodes and their eggs were present in large numbers in the pythium lesions.

Roots from the soil containing the nematodes alone, however, appeared normal in every respect and were almost entirely free from the nematodes; only one small necrotic spot in the processed roots was found to contain a few A. avenae. However, when soil from these pots was examined with the wet sieving technique, it was found to contain the nematodes mostly in the adult stage and somewhat starved in appearance. These facts, together with the healthy appearance of the roots, indicate that if the nematodes had fed on the roots the feeding was ectoparasitic and was not sufficiently effective to allow much reproduction of the nematodes.

Table 1. Oven dry weights of corn tops 4 weeks after planting.

Treatment	Mean weight, (grams)	Significance [a] 5 percent	1 percent
125,000 *A. avenae* per pot	4.50		
Control	4.49		
Pythium + 125,000 *A. avenae*	4.42		
Pythium + 50,000 *A. avenae*	3.52		
Pythium + *Pyrenochaeta* sp.	1.38		
Pythium alone	1.10		

[a]Any two means not bracketed by the same line are significantly different, and any means bracketed by the same line are not significantly different.

Table 2. Moist weights of roots after a 3-minute spin at 410 rpm in an automatic clothes' washer set in the spin-dry cycle.

Treatment	Mean weight, (grams)	Significance [a] 5 percent	1 percent
Control	26.44		
125,000 *A. avenae* per pot	23.10		
Pythium + 125,000 *A. avenae*	20.08		
Pythium + 50,000 *A. avenae*	18.66		
Pythium + *Pyrenochaeta* sp.	7.12		
Pythium alone	5.48		

[a]See footnote, Table 1.

DISCUSSION

Perhaps the chief significance of the results reported here is the information gained on the food habits of *Aphelenchus avenae*. If this nematode fed at all on the corn roots, it was essentially innocuous and ectoparasitic feeding, and there is no evidence that it fed at all except on mycelium. It is recognized that such a known ectoparasite as *Paratylenchus minutus* (13) would not have caused root or top symptoms under conditions such as these short-term tests, yet it has been the occurrence of *A. avenae* within plant tissues that has aroused the suspicion that *A. avenae* was a parasite of higher plants. In this test, the nematode freely invaded the roots that also were invaded by the pythium, and at the same time it greatly reduced the severity of root rot. From these facts it appears that the population of *A. avenae* used is not a root parasite of corn, but instead is a beneficial mycophagous nematode that tends to limit the development of a root-rotting fungus.

The numerous small brown lesions on roots exposed to pythium and the nematode together indicate that the nematodes were less effective in preventing fungal infection than in suppressing the fungus after it had begun to invade the root. Nematodes were absent from many of these small lesions. The meaning of this is not clear, yet many such lesions were very shallow and it is possible that the nematode had fed on the invading hyphae while its body lay chiefly on the root surface.

Despite the great magnitude of root rot control by the nematodes demonstrated here, the practical significance of the phenomenon should not be overestimated. Experimental conditions were very artificial. The potting mixture contained rotted manure that, after steaming, doubtless furnished nutrients for vigorous vegetative growth of the pythium. Numerous investigators have shown that related fungi exhibit more aggressive pathogenicity when added to a sterilized medium than when added to a natural soil, and various soil microorganisms have been shown to be antagonistic to pythium (9, 10, 14). Although A. avenae is found widely distributed in natural soils, it is not found in such great concentrations as were added to the experimental pot cultures in these studies. Determination of the significance of such nematodes in control of fungus root rots in nature remains to be achieved.

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NEMATODES ASSOCIATED WITH MINNESOTA CROPS II. NEMATODES ASSOCIATED WITH CORN, BARLEY, OATS, RYE, AND WHEAT[1]

Donald P. Taylor and E. Gordon Schleder[2]

Abstract

Nematodes were identified from 73 corn, 33 barley, 44 oats, 8 rye, and 18 wheat fields in Minnesota during the growing seasons of 1957 and 1958. The most commonly identified genera composed of only plant parasitic species and their percent of occurrence in samples collected were: Tylenchorhynchus, 59 percent; Helicotylenchus, 54 percent; Xiphinema, 52 percent; Pratylenchus, 50 percent; Hoplolaimus, 30 percent; and Paratylenchus, 27 percent. Other possible plant parasites encountered were species of Aphelenchoides, Aphelenchus, Boleodorus, Criconemoides, Ditylenchus, Gotholdsteineria, Heterodera, Meloidogyne, Neotylenchus, Nothotylenchus, Psilenchus, Tetylenchus, Trichodorus, Trophurus, and Tylenchus.

A survey of nematodes associated with Minnesota crops was initiated in 1956 as part of the nematode project of the Minnesota Agricultural Experiment Station. An earlier survey report (5) demonstrated that nematodes were much more commonly associated with crops in Minnesota than had been previously reported. The survey reported in this paper confirms this finding and increases the number of crops now known to have large nematode populations associated with them in the field.

During the growing seasons of 1957 and 1958, 176 soil samples were collected from Minnesota corn, barley, oats, rye, and wheat fields throughout the major farming areas of the State (Table 1).

Each sample is a composite of 10 to 20 subsamples taken from the lowest portions of fields or from areas where the plants grew poorly. A subsample consisted of a core of soil, including fibrous roots, 6 to 8 inches long collected from the rhizosphere of living plants with a soil sampling tube 1 inch in diameter. Soil samples were processed using a technique involving decanting, screening, and modified Baermann funnels. Nematodes were collected on #80 and #270 U. S. Standard Testing Sieves.

Stylet-bearing nematodes recovered from the funnels were relaxed with heat in a constant temperature oven and fixed in 3 percent formaldehyde. Specimens were placed on microslides and identified with a compound microscope equipped with an oil immersion objective.

Occurrence data for 21 genera of known or suspected plant parasitic nematodes are included in Table 2. Based on occurrence on the five crops reported, Tylenchorhynchus Cobb, 1913, Helicotylenchus Steiner, 1945, Xiphinema Cobb, 1913, Pratylenchus Filipjev, 1934, Hoplolaimus von Daday, 1905, and Paratylenchus Micoletzky, 1922 were the most frequently encountered genera believed to contain only plant parasites.

Corn: Large populations of several plant parasitic species were found in many of the 73 soil samples collected from corn fields. The frequencies of occurrences of Helicotylenchus, Pratylenchus, Tylenchorhynchus, and Xiphinema were higher than the all-crop figures for these genera. Species of Helicotylenchus (4), and Pratylenchus (3) are known to cause severe losses to corn in other States. This fact, together with the abundance of these genera in corn fields, suggests that Minnesota corn may be suffering unrecognized damage caused by nematodes.

The genus Trophurus Loof, 1955 was recovered in one sample from corn. The species encountered was probably the same as described by Caveness (2) as Clavaurotylenchus minnesotensis from sugar beets in Minnesota.

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Table 1. Distribution by county of soil samples from corn, barley, oats, rye, and wheat.

	Crop[a]							Crop					
	C	B	O	R	W	T[b]		C	B	O	R	W	T
Northwest District							**East Central District**						
Becker	1	4	-	-	1	6	Aitkin	-	-	1	-	-	1
Clay	-	2	-	-	-	2	Anoka	-	-	1	-	-	1
Kittson	-	4	3	-	-	7	Carlton	-	-	2	-	-	2
Mahnomen	-	2	-	-	1	3	Hennepin	-	-	1	1	1	3
Marshall	2	6	1	-	-	9	Kanabec	-	-	1	-	-	1
Norman	-	2	-	-	-	2	Mille Lacs	1	-	-	-	-	1
Pennington	-	1	-	-	-	1	Pine	-	-	3	-	-	3
Polk	-	7	-	-	2	9	Ramsey	-	-	1	-	-	1
Roseau	1	1	-	-	-	2	Washington	1	-	-	-	-	1
Total	4	29	4	-	4	41	Total	2	-	10	1	1	14
North Central District							**Southwest District**						
Itasca	-	-	1	-	-	1	Redwood	1	-	-	-	-	1
Total	-	-	1	-	-	1	Total	1	-	-	-	-	1
West Central District							**South Central District**						
Big Stone	2	-	-	-	1	3	Faribault	1	-	-	-	-	1
Chippewa	1	-	-	-	-	1	Freeborn	2	-	-	-	-	2
Douglas	1	1	2	-	-	4	LeSueur	2	-	1	-	2	5
Grant	-	-	2	-	1	3	Martin	1	-	-	-	-	1
Ottertail	-	2	1	-	-	3	Rice	4	-	1	-	-	5
Swift	1	1	3	-	-	5	Steele	3	-	2	-	1	6
Traverse	2	-	-	-	-	2	Waseca	2	-	1	1	1	5
Wilkin	-	-	-	-	1	1	Watonwan	-	-	-	-	1	1
Total	7	4	8	-	3	22	Total	15	-	5	1	5	26
Central District							**Southeast District**						
Benton	2	-	-	-	-	2	Dakota	3	-	3	1	2	9
Kandiyohi	2	-	1	-	-	3	Dodge	2	-	-	-	-	2
McLeod	3	-	-	-	-	3	Goodhue	4	-	-	-	-	4
Meeker	4	-	-	-	-	4	Mower	1	-	-	-	-	1
Morrison	4	-	2	-	-	6	Wabasha	-	-	1	-	-	1
Renville	7	-	-	-	-	7	Total	10	-	4	1	2	17
Scott	1	-	1	1	1	4							
Sherburne	-	-	-	1	-	1							
Stearns	4	-	5	2	1	12							
Todd	4	-	-	-	-	4							
Wright	3	-	3	1	1	8							
Total	34	-	12	5	3	54	Grand Total	73	33	44	8	18	176

[a]C = Corn, B = Barley, O = Oats, R = Rye, W = Wheat.
[b]Total samples per county.

Barley: The majority of barley soil samples was collected in the northwestern part of the State (Red River Valley) in 1958 where near drought conditions occurred throughout most of the growing season. Lack of moisture may have contributed to the small populations of most genera on barley. Of the six most common genera mentioned above, only Tylenchorhynchus occurred in barley fields in numbers approximating its all-crop figure. Paratylenchus, Pratylenchus, and Xiphinema were much less abundant than their average numbers for all crops.

Oats: Helicotylenchus, Hoplolaimus, Paratylenchus, Pratylenchus, Tylenchorhynchus, and Xiphinema occurred in oats samples about as frequently as their total occurrence. Xiphinema chambersi Thorne, 1939 was encountered in large numbers from one oats sample. This constitutes a first report of this infrequently seen species in Minnesota.

Rye: The small number of samples collected from this crop makes it difficult to draw conclusions. However, the high occurrence of Helicotylenchus spp. and Xiphinema americanum Cobb, 1913 should be noted.

Table 2. Nematodes associated with corn, barley, oats, rye, and wheat in Minnesota.

Nematodes	Crop plant: Corn (73)		Barley (33)		Oats (44)		Rye (8)		Wheat (18)		Total (176)	
	Number of samples: n[a]	p[b]	n	p	n	p	n	p	n	p	n	p
Aphelenchoides spp.	7	10	2	6	6	14	-	-	2	11	17	10
Aphelenchus avenae	50	68	22	67	33	75	4	50	13	72	122	69
Criconemoides sp.	-	-	1	3	-	-	-	-	1	6	2	1
Ditylenchus spp.	3	4	1	3	2	5	1	13	2	11	9	5
Gotholdsteineria sp.	1	1	1	3	1	2	-	-	-	-	3	2
Helicotylenchus spp.	10	14	4	12	-	-	-	-	1	6	15	9
H. erythrinae[c]	20	27	3	9	5	11	1	13	3	17	32	18
H. nannus[d]	19	26	5	15	16	36	6	75	6	33	52	30
Total[e]	45	62	12	36	21	48	7	88	10	55	95	54
Heterodera spp.	1	1	1	3	2	5	-	-	-	-	4	2
Hoplolaimus tylenchiformis	19	26	7	21	17	39	2	25	8	44	53	30
Meloidogyne sp.	2	3	1	3	-	-	-	-	-	-	3	2
Neotylenchidae (not identified)	9	12	2	6	7	16	2	25	3	17	23	13
Boleodorus spp.	44	60	9	27	19	43	2	25	8	44	82	47
Neotylenchus spp.	1	1	2	6	-	-	-	-	1	6	4	2
Nothotylenchus spp.	54	74	13	39	26	59	4	50	12	67	109	62
Paratylenchus spp.	20	27	3	9	21	48	1	13	2	11	47	27
Pratylenchus spp.	15	21	2	6	5	11	-	-	4	22	26	15
P. hexincisus	28	38	3	9	14	32	2	25	7	39	54	31
P. minyus	2	3	-	-	-	-	-	-	-	-	2	1
P. scribneri	1	1	1	3	1	2	-	-	1	6	4	2
P. penetrans	5	7	-	-	1	2	-	-	1	6	7	4
P. pratensis	2	3	-	-	-	-	-	-	-	-	2	1
Total	47	64	6	18	20	45	2	25	13	72	88	50
Psilenchus spp.	20	27	10	30	12	27	-	-	6	33	48	27
Tetylenchus sp.	3	4	2	6	-	-	-	-	-	-	5	3
Trichodorus sp.	2	3	-	-	1	2	-	-	-	-	3	2
Trophurus sp.	1	1	-	-	-	-	-	-	-	-	1	1

Table 2 (continued)

Nematodes	Crop plant: Corn (73) n[a]	Corn (73) p[b]	Barley (33) n	Barley (33) p	Oats (44) n	Oats (44) p	Rye (8) n	Rye (8) p	Wheat (18) n	Wheat (18) p	Total (176) n	Total (176) p
Tylenchorhynchus spp.	3	4	-	-	-	-	-	-	-	-	3	2
T. (fischeri)[f]	23	31	6	18	6	14	-	-	-	-	35	20
T. striatus	11	15	2	6	8	18	1	13	5	28	27	15
T. clarus	2	3	-	-	1	2	-	-	2	11	5	3
T. cylindricus	2	3	-	-	-	-	-	-	-	-	2	1
T. latus	-	-	3	9	-	-	-	-	1	6	4	2
T. maximus	-	-	-	-	1	2	-	-	-	-	1	1
T. acutus	10	14	4	12	8	18	-	-	3	17	25	14
T. nudus	10	14	4	12	3	7	-	-	4	22	21	12
Total	49	67	18	55	23	52	1	13	13	72	104	59
Tylenchus sp.	35	48	20	61	24	55	4	50	16	89	99	56
Xiphinema americanum	46	63	7	21	24	55	6	75	8	44	91	52
X. chambersi	-	-	-	-	1	2	-	-	-	-	1	1
Total	46	63	7	21	25	57	6	75	8	44	92	52

[a]Number of samples from which each form was recovered.
[b]Percentage of samples from which each form was recovered.
[c]Helicotylenchus erythrinae includes forms belonging to a closely related undescribed species.
[d]Helicotylenchus nannus includes forms belonging to a closely related undescribed species.
[e]Equals the number and percentage of samples positive for the genus. It will be lower than the sum of the individual species whenever a sample contains more than one species of a genus.
[f]Tylenchorhynchus fischeri Caveness (in press).

Wheat: Hoplolaimus tylenchiformis von Daday, 1905, Pratylenchus spp., and Tylenchorhynchus spp. were recovered much more frequently from soil samples collected from wheat fields than from all other crops reported. The occurrence of Pratylenchus in about 3/4 of wheat samples may indicate that considerable damage is being caused to this crop in Minnesota particularly when the pathogenic capabilities to wheat of root-lesion nematodes is considered (1).

Tests are in progress in this department to determine host ranges of many of the species frequently encountered in the survey. Additional studies on the pathogenicity to several crops of some of these species, alone and in combination with other soil-borne pathogens, are in progress.

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DISEASES OF CORN AND OF SORGHUM SPECIES IN INDIANA IN 1958[1]

A. J. Ullstrup and F. A. Laviolette[2]

The 1958 growing season was unusually cool; each month from January through September showed a deficiency in average temperature. Rainfall was less than normal for the first 5 months, but in June, July, and August excesses recorded in the vicinity of Lafayette were 7+, 5+, and 0.9 inches, respectively. September showed a deficiency of 1.6 inches. As a consequence of these weather conditions, the disease situation on corn and Sorghum spp. was somewhat different from that normally observed.

CORN DISEASES

Northern corn-leaf blight (Helminthosporium turcicum) was more prevalent throughout Indiana than it has been in any year since 1951. Distribution was spotty but generally more abundant in the southern half of the State. Some fields were badly blighted by the last week in August, and appreciable losses in yield may be expected in these fields. Weather conditions were ideal for the development of the disease. This fact, coupled with the abundance of inoculum that overwintered, provided favorable factors for initiation and spread of this blight.

Southern corn-leaf blight (H. maydis) was scattered over the State, and in some fields it had become severe by August 15. This was particularly true in some seed fields where susceptible inbred lines were grown.

Stalk rots are unusually prevalent in Indiana this year, with the rot incited by Gibberella zeae in greater abundance than generally observed. This pathogen seems to become relatively more prevalent in cool, moist seasons, whereas Diplodia zeae appears to be the predominant cause of stalk rot of corn when weather conditions during growing seasons approach those considered to be "normal" for Indiana. Actual stalk breakage had not become acute by the end of October owing largely to the absence of wind and rain storms.

Ear rots were not severe in 1958 except in a few very localized instances. Gibberella ear rot, normally of minor consequence in Indiana, was relatively more prevalent than it has been in several preceding years. Diplodia ear rot was not abundant during 1958 except for one localized area in southeastern Indiana. Nigrospora cob rot (Nigrospora oryzae) was widespread but not severe. Fusarium kernel rot was appreciably less abundant than usual.

Crazy top of corn, incited by Sclerophthora macrospora, was found in several areas of the State, but in none of the fields was there a large number of plants infected.

Common corn rust (Puccinia sorghi) was widespread, but severity did not approach the higher level observed in 1950 and 1951. Southern corn rust (P. polysora) was not seen in Indiana in 1958, although an intensive search for it was made in those areas where it was found in 1957[3].

Corn smut (Ustilago maydis) was somewhat more prevalent in 1958 than it has been in recent years. Susceptible inbred lines and hybrids showed an unusual amount of infection. A survey of the incidence of the disease was conducted over the major corn-growing areas of the State, which included an examination of 108 fields. In each field 10 samples of 100 plants each were observed and the percentage of infection as well as the size and location of galls was recorded. The incidence in the fields ranged from 0.5 percent to 9.0 percent, with an average of 2.4 percent. An estimate of the total loss was calculated by multiplying the yield reduction resulting from galls of different size and at different locations on the individual plant by the incidence. This is shown in Table 1.

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[1] Cooperative investigations, Crops Research Division, Agricultural Research Service, United States Department of Agriculture, and the Purdue University Agricultural Experiment Station.

[2] Pathologist, Department of Botany and Plant Pathology, Purdue University, and Crops Research Division, United States Department of Agriculture; and Instructor, Department of Botany and Plant Pathology, Purdue University, respectively.

[3] Ullstrup, A. J. 1958. The occurrence of southern rust and other corn diseases in Indiana in 1957. Plant Disease Reptr. 42: 373.

Table 1. The average percentage loss in yield sustained by individual plants in relation to size and location of smut galls; the incidence of the disease classes; and the calculated total yield loss attributable to each category.

Location and size of smut galls	Individual loss[a]	Incidence	Calculated total loss
	Percent		
Tassel galls	64.6	0.32	0.21
Large galls above ear	44.2	0.81	0.36
Small galls above ear	12.7	0.53	0.07
Large galls below ear	31.7	0.17	0.05
Small galls below ear	12.0	0.13	0.01
Ear galls	90.2	0.49	0.44
Total		2.4	1.1

a Based on data collected in 1957 (P.D.R. 42: 374-375, 1958).

DISEASES OF SORGHUM SPP.

Leaf blight, incited by Helminthosporium turcicum, was widespread on Sudan grass throughout Indiana and was moderately severe by August 15 on the susceptible varieties Piper, Common, and Sweet. Most of the leaves on these varieties had been destroyed by the disease by mid-September. Johnson grass was uniformly infected in southern Indiana, but very little of this disease was found on cultivated varieties of sorghum.

Bacterial stripe (Pseudomonas andropogonis) was present throughout the southern two-thirds of the State and moderately severe on the varieties Tx 74, Reliance, Hegari, and Manchuria Brown Kaoliang in the sorghum nursery of R. C. Pickett at Lafayette. The disease was appreciably less severe on the varieties of Sudan grass in this nursery.

Rust (Puccinia purpurea) was collected on Sudan grass, Johnson grass and a number of varieties of sorghum, but on only a few forage sorghums did it become established in severe proportions before frost.

Head smut (Sphacelotheca reiliana) was found in only one instance, this being a single infected plant in a field bordering on the Ohio River in southeastern Indiana.

FIGURE 1. Crazy top of of sorghum. Note stunting, tillering, lighter color of leaves, and failure to produce a head on the plant in the foreground.

Crazy top of sorghum and Sudan grass was observed in several fields in the State, but the number of plants infected in any one field was small. In all areas where infected plants were found there was evidence of water-logging of the soil. Infected plants were stunted and excessively tillered; leaves were narrow, strap-like, thick and leathery in texture, and light green in color (Figure 1). In most instances heads failed to develop or were barren, but in a few plants proliferation of the floral structures was similar to that found in other hosts. An abundance of oospores was observed in the leaf tissue, and from their size, shape and color it appeared that the inciting agent was Sclerophthora macrospora. This report is the first of the occurrence of the disease in Indiana on these hosts.

A number of minor leaf diseases were abundant on Johnson grass and wild cane in the Ohio River bottoms. These were zonate leaf spot (Gloeocercospora sorghi), gray leaf spot (Cercospora sorghi), sooty stripe (Ramulispora sorghi), and rough spot (Ascochyta sorghina). These diseases were of no consequence on the cultivated varieties of sorghum and Sudan grass, nor were they abundant outside river bottoms in extreme southern Indiana. These leaf diseases have not been reported previously in Indiana. It should be pointed out, however, that J. Tuite and M. Britton found and identified rough spot on a volunteer plant of Johnson grass on the campus of Purdue University in 1957.

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A NON-PARASITIC LEAF SPOT OF OATS[1]

A. L. Hooker[2]

Abstract

Field and greenhouse studies indicate that a non-parasitic leaf spot develops on certain oat varieties when the environment in in which they are growing changes from cool and moist to warm and dry. Rapid drying of water-congested leaf tissue appears to be particularly favorable for inducing symptom expression.

Non-parasitic leaf spots and leaf necroses of various types occur on certain oat varieties. Spots due to manganese deficiency (2, 4, 5, 6) and to genetic factors (3) and terminal bleaching due to hot, dry winds (7) have been described in the literature. Chester (1), working with rust, observed the development of necrotic leaf spots on certain hybrid wheat selections when they were repeatedly subjected to 100 percent humidity at 18° C for 12 hours nightly. These spots were preceded by water-soaked lesions from which the hybrids did not recover and were similar to a "physiologic leaf spot" which developed in the field. Chester indicated that a similar spot occurred on the leaves of Kareela oats.

The purpose of the present study was to determine some of the causal factors associated with non-parasitic leaf spots on oat leaves.

FIELD OBSERVATIONS

Several oat varieties observed by various workers to express so-called "non-parasitic" leaf spots were collected. These and check varieties were grown in several plots at Madison, Wisconsin in 1956 and 1957 and were observed frequently during the growing seasons.

Leaf spots developed on the Craig (C.I.[3] 5332), Kareela (C.I. 2774), G.A. 50 (C.I. 7436), and La Estanzuela (C.I. 7049) varieties but not on the resistant varieties Clinton (C.I. 3971) and Clintafe (C.I. 5869). Spots also developed on the variety Burnett (C.I. 6537). The symptoms on G.A. 50 (Fig. 1) were typical of those observed on the other varieties. G.A. 50 is a selection from the cross Erban x Boone.

Leaf spots were first observed on June 15 in 1956 (Table 1). This followed 6 days of warm, dry weather preceded by a cool and moist period in June (Fig. 2) and above normal rainfall in May.

Leaf spots were later in appearance and less severe in 1957 (Table 1). Weather conditions were more variable in 1957 (Fig. 3); but, as in 1956, the leaf spots on G.A. 50 developed following a warm, dry period preceded by cloudy, cool and humid conditions.

GREENHOUSE EXPERIMENTS

Following the field observations in 1956, several greenhouse experiments in which resistant and susceptible oat varieties were subjected to various environmental conditions and changes were conducted. As an environmental change from cool and moist to warm and dry was associated with symptom development in the field, this environmental change was studied more extensively than others.

Results from one experiment, representative of the preliminary tests, are summarized in Table 2. In this experiment, plants of five oat varieties were grown for 5 weeks at approx-

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[1]Contribution from the Crops Research Division, Agricultural Research Service, United States Department of Agriculture, and the Department of Plant Pathology, Wisconsin Agricultural Experiment Station, Madison, Wisconsin. Published with the approval of the Director of the Wisconsin Agricultural Experiment Station.

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[3]C.I. refers to accession number of the Crops Research Division.

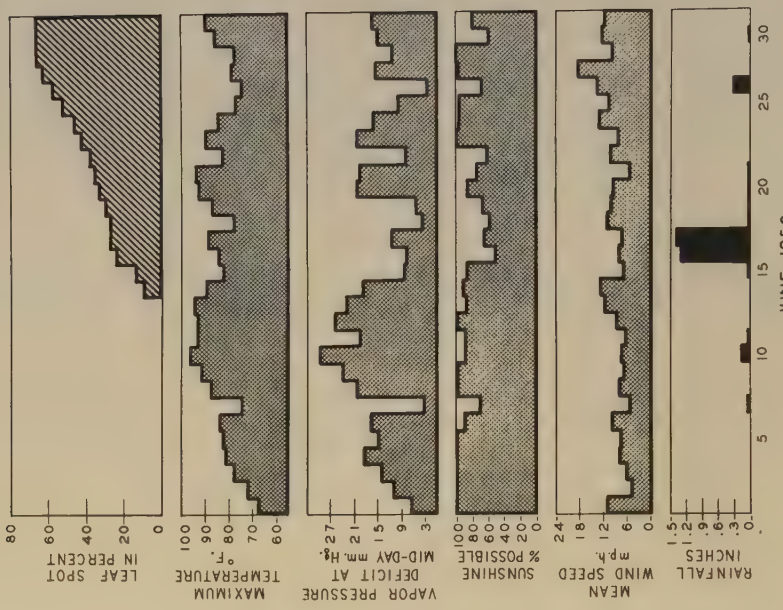

FIGURE 2. Interrelation of symptoms of non-parasitic leaf spot on G.A. 50 oats and weather conditions at Madison, Wisconsin, June 1956.

FIGURE 1. Symptoms of non-parasitic leaf spot on G.A. 50 oats in the field at Madison, Wisconsin.

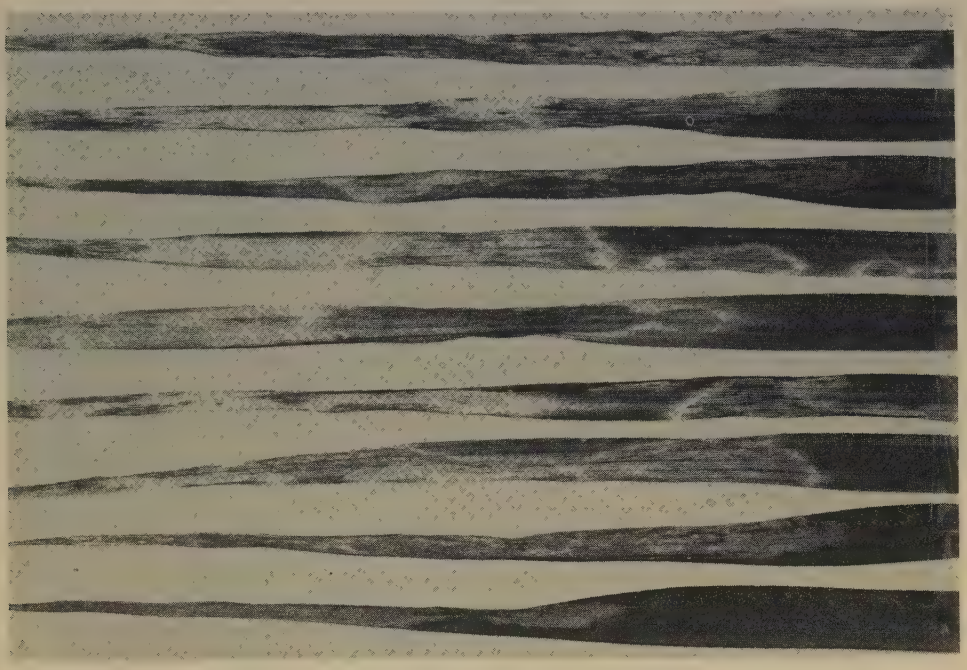

FIGURE 4. Symptoms of non-parasitic leaf spot induced on G. A. 50 oats in the greenhouse by moving plants from a cool, moist environment to a warm, dry environment.

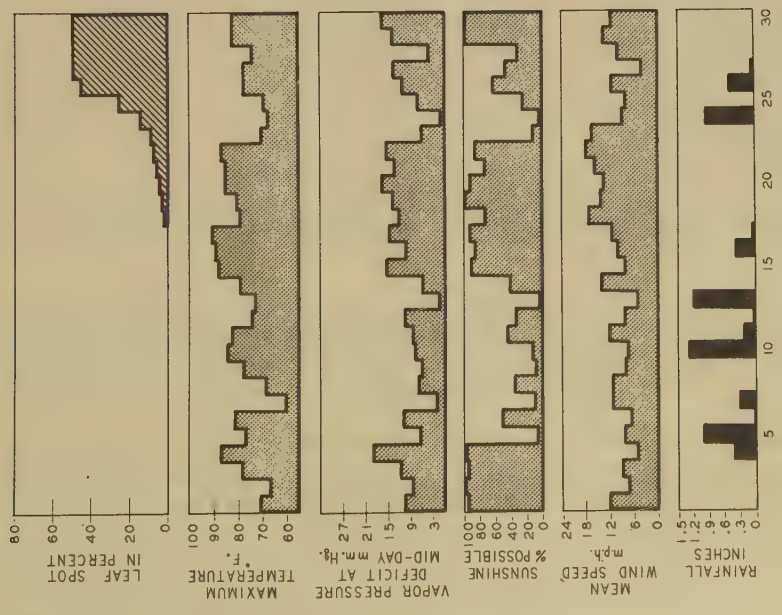

FIGURE 3. Interrelation of symptoms of non-parasitic leaf spot on G. A. 50 oats and weather conditions at Madison, Wisconsin, June 1957.

Table 1. Percentage of leaf area covered with non-parasitic leaf spots on five oat varieties on various dates and dates of heading, in the field, Madison, Wisconsin, 1956 and 1957.

Year and oat variety	Heading date	Percentage leaf spot on: June 7	June 15	June 18	June 21	June 26	June 28	July 3	July 9
1956:									
Clinton	June 19	0	0		0		0		
Craig	24	0	20		25		30		
Kareela	20	0	25		30		30		
G.A. 50	18	0	25		35		65		
La Estanzuela	25	0	30		30		30		
1957:									
Clinton	June 29			0		0		0	0
Craig	July 3			0		3		5	5
Kareela	June 29			0		4		10	10
G.A. 50	27			5		45		50	50
La Estanzuela	July 7			0		4		5	8

Table 2. Percentage of leaf area covered with non-parasitic leaf spots on five oat varieties grown at 16° C for 5 weeks in the greenhouse and then subjected to seven environmental changes for 10 days. Average of three replications.

Environmental change	Clinton	Craig	Kareela	G.A. 50	La Estanzuela
16° C (no change)	0	0	0	0	0
16° C to 20° C	0	0	0	3	0
16° C to 24° C	0	0	0	6	0
16° C to 28° C	0	2	1	9	0
16° C to MC[a] to 16° C	0	0	1	3	0
16° C to MC to 20° C	0	0	1	6	0
16° C to MC to 24° C	0	0	4	18	0
16° C to MC to 28° C	0	2	5	48	1

[a]MC=Moist chamber and wet soil for 24 first hours of 10-day period.

imately 16° C. They were then subjected to the environmental changes listed in Table 2. Leaf spots developed on the leaves of G.A. 50 and several other varieties within 10 days following the shift in environments. No spots developed on the resistant variety Clinton or on plants kept at 16° C for the entire period. The symptoms on G.A. 50 (Fig. 4) were similar to those observed in the field.

These data were confirmed in a series of four separate experiments summarized in Table 3. These experiments were started in September, and in some tests daily fluctuations in temperature occurred in the cool greenhouse in which the plants were established. Results from all experiments, however, were essentially alike. Symptom expression was greatest on leaves of G.A. 50 which had been subjected to environmental changes. They were particularly severe on plants placed in a moist chamber for 12 hours and then rapidly dried by warm air moving over the foliage (fan treatment at 28° C). In some experiments, a few spots developed on the check plants maintained in the cool greenhouse for the entire period.

The relationship of time period at 28° C and the influence of manganese sulfate treatment to symptom production was studied in another experiment (Table 4). Plants of the susceptible variety G.A. 50 and of the resistant variety Clintafe were grown at approximately 16° C for 40 days, placed in a moist chamber for 24 hours, and then incubated at 28° C for periods of

Table 3. Percentage of leaf area covered with non-parasitic leaf spots on five oat varieties grown at approximately 16° C for 30-45 days and then subjected to five environmental changes for 10 days. Average of four experiments with two replications each.

Environmental change	Clintafe	G.A. 50	La Estanzuela	Kareela	Craig
16° C[a] (No change)	0.0	6.5	0.0	1.9	0.0
16° C to MC[b] to 28° C	0.0	32.5	0.0	0.4	7.0
16° C to MC to 16° C	0.0	7.9	0.0	0.6	0.4
16° C to MC to 28° C[c]	2.5	51.6	2.9	2.0	9.0
16° C to 28° C	0.0	34.3	1.9	2.6	7.1
16° C to 16° C[d]	0.0	12.4	0.0	1.0	0.6

[a]Approximately 16° C.
[b]MC=Moist chamber and wet soil for first 12 hours of 10-day period.
[c]Placed 6 feet in front of 18-inch fan operating at slow speed for 2 hours immediately after removal from moist chamber to promote rapid drying of foliage.
[d]Separate greenhouse unit operating at nearly constant temperature.

Table 4. Interrelation of period at 28° C, treatment with manganese sulfate, and development of non-parasitic leaf spot on G.A. 50 oats.

Period at 28° C (hours)	Percentage leaf spot: Without manganese sulfate	Percentage leaf spot: With manganese sulfate[a]
0	3	3
8	20	20
24	35	30
32	25	30
48	35	35
56	40	35
72	40	40
80	55	50
120	65	65
128	70	60
144	70	70
Check[b]	2	2

[a]Manganese sulfate (0.4 percent solution) applied as a foliage spray and soil drench 31 days after planting.
[b]Maintained at approximately 16° C.

0 to 144 hours. The plants were then returned to the cool greenhouse. Percentages of non-parasitic leaf spot were recorded for all treatments 8 days after the moist-chamber incubation. Symptoms developed only on G.A. 50. They developed with only 8 hours at 28° C but were more severe as the length of incubation at 28° C increased. Manganese sulfate applied as a foliage spray and soil drench had little influence on symptom production.

As young plants were employed in most of the greenhouse tests, and in some cases a few spots developed on plants not intentionally subjected to environmental changes, attempts were made to grow plants under a more uniform environment for a longer period. Plants of G.A. 50 were established in an air-conditioned greenhouse operating at a nearly constant temperature of 18° C. They were observed through heading to the soft dough stage. No symptoms developed on these plants, although plants of a similar age had always shown leaf spot symp-

toms in the field (Table 1).

ISOLATION ATTEMPTS

Attempts to isolate a pathogenic organism from the leaf spots were unsuccessful. In addition, known parasitic leaf diseases were not in evidence when this condition first appeared each season in the field and, of course, were not present in the greenhouse.

DISCUSSION

Non-parasitic diseases often are very difficult to study as the causal factor or factors frequently occur at some undetermined time prior to symptom expression.

The data obtained in this study may not be conclusive, but it appears that a non-parasitic leaf spot develops on certain oat varieties as the result of specific environmental changes. The condition is similar to, but probably not identical with, that described by Chester (1).

Field and greenhouse studies indicate that this condition develops in certain oat varieties when plants are exposed to a change from a cool and moist environment to one that is warm and dry. Rapid drying of water-congested leaf tissue appears to be particularly favorable for inducing symptom expression.

Not all oat strains which have expressed so-called "non-parasitic" leaf spots in the field, however, have expressed them to the same degree in the greenhouse studies made. Conceivably, further research will reveal other causal factors to be involved and that the environmental changes merely accelerate symptom expression.

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CO-OPERATIVE SEED TREATMENT TRIALS -- 1958[1]

J. E. Machacek[2]

Abstract

Nineteen new or comparatively new seed dressings were tested in 1958 against bunt of wheat (mixed Tilletia foetida (Wallr.) Liro and T. caries (DC.) Tul.), smut of oats (mixed Ustilago avenae (Pers.) Rostr. and U. kolleri Wille), covered smut of barley (U. hordei (Pers.) Lagerh.), and against seed rot of flax resulting from threshing injury. These tests were carried out under experimental plot conditions at a number of stations in the United States and Canada. The plot tests were supplemented by tests in the greenhouse at Winnipeg to show the degree to which the seed dressings controlled seedling blight in wheat, oats, and barley. The plot tests showed that all but one of the seed dressings gave good control of wheat bunt, that none gave good control of oat smut, and that the control of barley smut and flax seed rot was variable. The control of seedling blight in the greenhouse was also variable. The oat seed used for these tests appeared to be very sensitive to the chemical action of some of the dressings tested, as storage of seed treated with the dressings for 4 months in closed glass jars greatly reduced germination of the pathogen, especially where the dressings were liquids. The oat tests showed also that seed inoculation with smut spores applied by the partial-vacuum method resulted in much smut in the crop, even where the seed was treated with usually effective dressings. The amount of smut in oats was comparable to that obtained in previous years by the homogenizer (Blendor) method of seed inoculation.

MATERIALS AND METHODS

The seed dressings compared in 1958 were:

Buckman BSM-11 -- A liquid containing 50.0% of potassium 2,4,6-trichlorophenate and 6.0% mercury as phenyl mercury acetate. Obtained from Buckman Laboratories, Inc., Memphis, Tennessee.

Ceresan M -- A powder containing 3.2% mercury as ethyl mercury p-toluene sulfonanilide. Obtained from E. I. duPont de Nemours, Wilmington, Delaware.

Ceresan 100 -- A liquid containing 2.26% mercury as a mixture of ethyl mercury 2,3-dihydroxy propyl mercaptide and ethyl mercury acetate. Obtained from E. I. duPont de Nemours, Wilmington, Delaware.

Chipman BB-68 -- A liquid containing 1.5% mercury as methyl mercury nitrile. Obtained from Chipman Chemicals Ltd., Winnipeg, Manitoba.

Co-op. Liquid Mercury Concentrate -- A liquid containing 12.5% mercury as phenyl mercury acetate. Obtained from Interprovincial Co-operatives Ltd., Winnipeg, Manitoba.

Dieldrisan -- A powder containing 20.0% dieldrin and 1.25% mercury as mixed phenyl mercury acetate and ethyl mercury chloride. Obtained from Leytosan (Canada) Ltd., Winnipeg, Manitoba.

Dual Combination with Anti-friction Additive -- A powder containing 40.0% aldrin and 2.0% mercury as mixed phenyl mercury acetate and ethyl mercury chloride. Obtained from Gallowhur Chemicals Canada Ltd., Montreal, Quebec.

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[1] Contribution No. 16 from the Canada Department of Agriculture Research Laboratory, Winnipeg, Manitoba.

[2] Senior Plant Pathologist, Plant Pathology Section, Canada Department of Agriculture Research Laboratory, Winnipeg, Manitoba.

Gallotox 50 -- A liquid containing 2.88% mercury as phenyl mercury acetate. Obtained from Gallowhur Chemicals Canada Ltd., Montreal, Quebec.

Isotox - PMA -- A powder containing 45.0% lindane and 1.0% mercury as phenyl mercury acetate. Obtained from Ortho Agricultural Chemicals Ltd., Vancouver, British Columbia.

Mer-cad -- A powder containing 3.8% mercury as phenyl mercury formamide. Obtained from Stauffer Chemical Company, Omaha, Nebraska.

Mer-sol 7 -- A liquid containing 4.0% mercury as phenyl mercury ammonium acetate. Obtained from Stauffer Chemical Company, Omaha, Nebraska.

Mer-sol 51 -- A liquid containing 2.6% mercury as mixed phenyl mercury acetate and ethyl mercury acetate. Obtained from Stauffer Chemical Company, Omaha, Nebraska.

Metasan M -- A powder containing 3.2% mercury as methyl mercury 8-hydroxyquinolinate. Obtained from Natural Products Corporation, Montreal, Quebec.

Metasol M -- A liquid containing 1.5% mercury as methyl mercury 8-hydroxyquinolinate. Obtained from Natural Products Corporation, Montreal, Quebec.

New Gallotox -- A liquid containing 3.9% mercury as phenyl mercury acetate. Obtained from Gallowhur Chemicals Canada Ltd., Montreal, Quebec.

New Puradrin -- A powder containing 40.0% aldrin and 1.85% mercury as phenyl mercury formamide. Obtained from Gallowhur Chemicals Canada Ltd., Montreal, Quebec.

Ortho LM -- A liquid containing 1.35% mercury as methyl mercury 8-hydroxyquinolinate. Obtained from California Spray-Chemical Corporation, Maryland Heights, Missouri.

Ortho LME -- A liquid containing 1.35% mercury as mixed ethyl mercury 8-hydroxyquinolinate and methyl mercury 8-hydroxyquinolinate. Obtained from California Spray-Chemical Corporation, Maryland Heights, Missouri.

Panogen 15 -- A liquid containing 1.5% mercury as methyl mercury dicyan diamide. Obtained from Panogen Company, Ringwood, Illinois.

Smut-B-Gon -- A liquid containing 4.0% mercury as phenyl mercury acetate. Obtained from California Spray-Chemical Corporation, Maryland Heights, Missouri.

Triasan -- A powder containing 25.0% hexachlorobenzene and 50.0% auramine salt of dimethyldithiocarbamic acid. Obtained from Standard Chemical Limited, Winnipeg, Manitoba.

The seed used in the 1958 trials was as follows: wheat of the variety Red Bobs with the seed artificially contaminated with dry bunt spores (1:200, by weight); oats of the variety Vanguard with the seed artificially contaminated with a water-suspension of spores (equivalent to a spore load of 1:600) applied by the partial-vacuum method; barley consisting of a mixture of varieties (farm sample) and carrying a very heavy natural-load of smut spores; and flax of the variety Rocket with about 50 percent of the seed cracked during threshing.

The seed dressings were applied at the rates suggested by the manufacturers. When a rate was not given, the dressing was subjected to preliminary laboratory and greenhouse tests designed to show the rate to be used.

The seed and field plans for the main co-operative seed treatment trials were prepared at Winnipeg and sent to the co-operators (names and locations given at the end of this report) in time to reach them before the optimum date of sowing for their areas. Later, the data collected from the co-operators were analysed at Winnipeg and a summary prepared. Secondary tests, dealing with the control of seedling blight and with the effect of the various dressings on seed germination, were carried out in the greenhouse at Winnipeg only.

EXPERIMENTAL RESULTS

Table 1 is a summary of the experimental plot data obtained at Winnipeg and the co-operating stations. This summary shows that all of the seed dressings tested, except Triasan, gave good control of wheat bunt. None of the seed dressings tested gave good control of oat smut, although some gave better control than others. This result with oats is thought due, in part at least, to the method (partial-vacuum) used for seed inoculation. In another test with the same seed dressings, but using dry seed inoculated with dry spores, there was a much better differentiation of fungicides with respect to oat-smut control. The experimental plot data for cov-

Table 1. Summary of field data from the cooperative seed treatment trials of 1958.

Treatments	Dose (oz./bu.)				Smut (%)[a]			Germination (%)[b]
	Wheat	Oats	Barley	Flax	Wheat	Oats	Barley	Flax
Untreated dry seed	0.0	0.0	0.0	0.0	21.2	49.4	33.8	18.6
Powders								
Ceresan M	0.5	0.5	0.5	1.5	0.1	34.3	3.7	30.8
Canuck Mercury-Aldrin	2.0	1.4	1.4	5.0	0.2	36.8	3.4	28.8
Dieldrisan	2.5	2.5	2.5	2.5	0.1	36.2	8.9	23.1
Isotox-PMA	2.0	1.4	1.4	5.0	0.1	36.2	11.2	23.4
Mer-cad	0.5	0.5	0.5	1.5	0.1	38.7	9.9	22.6
Metesan M	0.5	0.5	0.5	1.5	0.1	31.2	6.7	29.5
"New" Puradrin	2.0	1.4	1.4	5.0	0.2	40.1	8.3	24.7
Triasan	0.5	0.5	0.5	1.5	6.0	44.4	29.3	23.8
Liquids								
Panogen 15	0.75	0.75	0.75	1.5	0.3	37.5	21.0	31.8
Buckman BSM-11	0.75	0.75	0.75	2.25	0.1	41.0	14.4	28.1
Ceresan 100	0.5	0.5	0.5	1.5	0.2	38.5	18.5	34.3
Ceresan 100	0.75	0.75	0.75	2.25	0.1	36.4	16.0	43.1
Chipman BB-68	0.75	0.75	0.75	1.5	0.1	38.0	13.5	31.1
Co-op. Liquid Mercury Concentrate[c]	0.75	0.75	0.75	1.5	0.2	44.8	7.5	28.9
Gallotox 50	0.75	0.75	0.75	1.5	0.1	41.3	14.3	27.3
Mer-sol 7	0.5	0.5	0.5	1.5	0.3	41.5	28.5	24.2
Mer-sol 7	0.75	0.75	0.75	2.25	0.2	40.0	21.8	29.6
Mer-sol 51	0.5	0.5	0.5	1.5	0.1	39.9	15.4	28.0
Mer-sol 51	0.75	0.75	0.75	2.25	0.1	38.4	19.8	32.7
Metasol M	0.75	0.75	0.75	1.5	0.2	37.4	20.1	32.3
"New" Gallotox	0.75	0.75	0.75	1.5	0.2	40.3	24.5	31.6
Ortho IM	0.75	0.75	0.75	1.5	0.2	37.9	20.4	27.7
Ortho LME	0.75	0.75	0.75	1.5	0.2	38.8	20.1	29.1
Smut-B-Con	0.5	0.5	0.5	1.5	0.1	43.2	28.3	24.6
Significant difference (5%)					1.2	3.2	3.0	3.7

[a] Smut data shown for wheat, oats, and barley are averages for 12 stations, 11 stations, and 16 stations respectively.

[b] Germination data for flax are averages for six stations.

[c] Concentrate diluted before use by adding two volumes of water to one volume of concentrate.

Table 2. Effect of storage on the germination of treated seed used in the co-operative seed treatment trials of 1958.

Treatment	Dose (oz./bu.)				Germination (%) after indicated duration of storage (months)							
					Wheat		Oats		Barley		Flax	
	Wheat	Oats	Barley	Flax	0	4	0	4	0	4	0	4
Untreated dry seed	0.0	0.0	0.0	0.0	61.0	54.0	65.0	30.0	29.5	31.0	14.0	32.5
Powders												
Ceresan M	0.5	0.5	0.5	1.5	73.5	70.0	75.0	59.0	57.0	42.5	49.0	55.5
Canuck Mercury - Aldrin	2.0	1.4	1.4	5.0	71.5	62.5	73.0	63.5	60.5	34.0	46.5	40.5
Dieldrisan	2.5	2.5	2.5	2.5	74.5	72.0	78.0	67.5	59.0	36.5	33.5	45.5
Isotox - PMA	2.0	1.4	1.4	5.0	60.0	58.0	51.0	44.0	52.0	45.5	34.5	36.0
Mer-cad	0.5	0.5	0.5	1.5	65.0	71.5	70.5	52.0	56.5	41.0	29.5	33.0
Metasan M	0.5	0.5	0.5	1.5	67.5	66.5	76.0	44.0	51.5	40.0	40.0	52.5
"New" Puradrin	2.0	1.4	1.4	5.0	67.5	72.5	73.5	63.0	46.0	39.5	38.5	52.0
Triasan	0.5	0.5	0.5	1.5	55.0	48.5	63.0	14.0	52.0	38.5	17.0	30.5
Liquids												
Panogen 15	0.75	0.75	0.75	1.5	74.5	74.0	63.0	13.5	55.0	48.0	35.5	39.0
Buckman BSM-11	0.75	0.75	0.75	2.25	68.0	30.5	72.0	1.5	50.5	42.5	51.0	53.0
Ceresan 100	0.5	0.5	0.5	1.5	66.5	57.5	74.0	5.5	55.5	47.5	35.0	56.5
Ceresan 100	0.75	0.75	0.75	2.25	71.5	49.0	71.5	5.5	56.5	35.5	54.0	56.0
Chipman BB-68	0.75	0.75	0.75	1.5	73.0	71.0	64.0	0.0	53.0	38.0	43.5	45.0
Co-op. Liquid Mercury Concentrate	0.75	0.75	0.75	1.5	67.0	68.5	70.0	9.0	54.5	55.5	39.0	44.5
Gallotox 50	0.75	0.75	0.75	1.5	65.5	60.5	79.0	2.0	48.5	44.5	40.5	51.0
Mer-sol 7	0.5	0.5	0.5	1.5	67.0	64.5	82.5	1.5	49.5	43.0	40.5	46.5
Mer-sol 7	0.75	0.75	0.75	2.25	65.5	66.5	75.5	2.0	57.5	43.0	43.5	45.0
Mer-sol 51	0.5	0.5	0.5	1.5	75.0	64.0	77.0	0.5	65.0	39.0	40.5	53.5
Mer-sol 51	0.75	0.75	0.75	2.25	74.0	68.5	70.0	0.0	48.0	43.5	47.5	53.5
Metasol M	0.75	0.75	0.75	1.5	69.0	82.5	63.0	1.5	53.5	32.5	44.0	40.0
"New" Gallotox	0.75	0.75	0.75	1.5	73.5	71.0	74.0	2.0	53.0	42.5	34.5	49.5
Ortho LM	0.75	0.75	0.75	1.5	72.5	68.0	78.5	5.0	46.0	44.5	33.0	45.5
Ortho LME	0.75	0.75	0.75	1.5	73.0	66.5	72.0	0.5	58.5	45.5	33.0	41.0
Smut-B-Gon	0.5	0.5	0.5	1.5	58.5	69.0	72.5	3.5	50.0	43.5	38.5	44.0
Significant difference (5%)					10.1	11.9	11.9	8.6	14.6	DNS[a]	10.7	10.4

[a]DNS = Differences not significant.

ered smut of barley showed fair control for some seed dressings and poor control for others, especially where the dressings were liquids. This poor control of barley smut may have been the result of the very heavy natural load of spores and spore aggregates carried by the seed. Control of flax seed rot ranged from fair to good, with Ceresan 100, applied at 2.25 ounces per bushel, giving the best results.

Table 2 is a summary of germination data obtained under greenhouse conditions. The untreated and treated wheat, oats, barley and flax seed was sown within a week of treatment, and again after 4 months of storage in closed glass jars. Data from the first sowing show that all the seed dressings except Isotox - PMA and Triasan improved germination in wheat, and that most of them also improved germination of oats, barley, and flax. Data from the second sowing showed that Buckman BSM-11 and Ceresan 100 (.75 ounces:bushel rate) were the only dressings that depressed germination of wheat. All the liquid dressings, and one powder dressing (Triasan), depressed germination of oats, in some instances very severely. Such injury did not occur in barley or flax. The reason for the low germination of treated, stored oat seed is not known but it is probably due to an unusually high susceptibility to chemical treatment of the seed lot used.

The writer wishes to thank the following workers for their help with the Co-operative seed treatment trials of 1958: Dr. M. C. Shurtleff (Ames, Iowa); Mr. A. A. Guitard (Beaverlodge, Alberta); Mr. W. H. Johnston (Brandon, Manitoba); Dr. J. F. Hennen (Brookings, South Dakota); Mr. J. E. Campbell (Charlottetown, Prince Edward Island); Dr. W. E. Brentzel (Fargo, North Dakota); Mr. W. A. Hodgson (Fredericton, New Brunswick); Dr. W. Crosier (Geneva, New York); Dr. S. G. Fushtey (Guelph, Ontario); Dr. R. I. H. McKenzie (Indian Head, Saskatchewan); Dr. M. L. Kaufmann (Lacombe, Alberta); Mr. J. S. Horricks (Lethbridge, Alberta); Dr. D. C. Arny (Madison, Wisconsin); Mr. H. R. Ballantyne (Melfort, Saskatchewan); Mr. W. J. Breakey (Morden, Manitoba); Mr. W. Bell (Ottawa, Ontario); Dr. L. H. Purdy (Pullman, Washington); Dr. L. O. Lachance (Ste. Anne de la Pocatière, Quebec); Dr. R. C. Russell and Prof. T. C. Vanterpool (Saskatoon, Saskatchewan); Mr. A. G. Kusch (Scott, Saskatchewan); Mr. D. S. McBean (Swift Current, Saskatchewan); and Mr. H. A. H. Wallace (Winnipeg, Manitoba).

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SORGHUM SEED-TREATMENT TESTS IN 1958[1]

O. J. Webster and R. W. Leukel[2]

In 1958 field tests were conducted to test the relative merits of a number of mercurial and nonmercurial fungicides on glume-free seed of Combine-60 kafir and on glumed seed of Rancher sorgo with regard to their effect on germination, field stands, and control of covered kernel smut, Sphacelotheca sorghi (Lk.) Clint.

Clean seed of Combine-60 kafir was artificially infested with spores of covered kernel smut. Seed lots of 500 cc (1/70 of a bushel by volume) were treated in 1-quart glass jars several weeks before planting. Germination tests were made on blotters, and emergence counts of plants grown in clean soil in the greenhouse at 75° F were made.

Later, in field plantings at Beltsville, Maryland, and Lincoln, Nebraska, a counted number of seeds of each seed lot was planted per row in several replications, and the seedlings per row were counted. After the plants were sufficiently mature to take data on infection, the total heads and the smutted heads in each row were counted. All data obtained are presented in Table 1.

Table 1. Germination, emergence, field stand and control of covered kernel smut in Combine-60 kafir, grown from smut-infested seed, treated as shown, and planted in field plots at Beltsville, Maryland, June 4, 1958, and at Lincoln, Nebraska, May 16, 1958.

Seed treatment applied				Beltsville				Lincoln		
No.	Fungicide	oz/bu	form	Percent germination and emergence (a)[a] (b)[b]		Percent field stand	Percent smutted heads	Percent germination	Percent field stand	Percent smutted heads
1	Untreated	-	-	88	68	44	65.8	80	36	4.0
2	Arasan 15	2	slurry	96	93	67	2.1	82	53	0
3	Orthocide	2	"	90	88	66	1.1	82	73	0
4	Panoram	2	"	90	89	79	0.4	84	68	0
5	Phygon	2	"	92	88	68	0	78	58	0
6	Spergon	2	"	92	70	59	0	88	53	0
7	Arasan S.F.M.	2	"	92	91	75	1.3	80	61	tr
8	Delsan	2	"	90	90	75	2.4	90	78	0
9	Vancide	2	liquid	94	86	64	11.9	90	33	tr
10	Copper carbonate	2	dust	92	77	61	2.7	92	60	0
11	Untreated	-	-	84	74	59	42.9	80	38	3.3
12	Ortho seed guard	2	slurry	92	74	78	0.6	78	80	tr
13	Panoram D-31	"	"	92	89	71	0.6	70	75	tr
14	Agrox	3/4	"	88	80	64	0.3	84	35	0
15	Ceresan M	3/4	"	96	90	60	1.2	84	47	0
16	Puraseed	3/4	"	88	80	60	0	84	36	0
17	Panogen	3/4	liquid	90	85	67	0	86	60	tr
18	Chipcote	3/4	"	96	72	51	0	86	66	0
19	Setrete	3/4	"	94	76	62	0	84	39	0.1
20	Ceresan 100	3/4	"	96	87	74	0	82	58	0

[a]On blotters at 75° F.
[b]In soil at 75° F.

At Beltsville soil conditions after planting were highly favorable for infection, which averaged 54.4 percent in the checks. Phygon, Spergon, and five mercurials eliminated smut infection completely. The other treatments with one exception reduced infection to less than 3 percent. Vancide, a nonmercurial liquid, allowed 11.9 percent infection (Table 1).

[1]Cooperative investigations, Nebraska Agricultural Experiment Station and Crops Research Division, Agricultural Research Service, United States Department of Agriculture.
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Table 2. Control of covered kernel smut in Rancher sorgo grown from infested seed, treated as shown and planted in field plots at Beltsville, Maryland and at Lincoln, Nebraska, 1958.

Seed treatment applied				Beltsville		Lincoln	
No.	Fungicide	oz/bu	form	Percent germination	Percent smutted heads	Percent field stand	Percent smutted heads
1	Check	-	-	90	32	66	8.2
2	Arasan 15	2	slurry	95	6	80	0
3	Orthocide	2	"	93	20	81	0
4	Panoram	2	"	94	9	86	0
5	Phygon	2	"	83	0	66	0
6	Spergon	2	"	92	0	78	0
7	Arasan S.F.M.	2	"	92	6	77	0
8	Delsan	2	"	97	21	82	0.5
9	Vancide	2	liquid	97	16	69	2.8
10	Copper carbonate	2	dust	97	6	70	0
11	Check	-	-	94	42	76	6.6
12	Ortho Seed Guard	2	slurry	89	40	77	1.0
13	Panoram D-31	2	"	97	11	76	0.2
14	Agrox	3/4	"	90	0	74	0
15	Ceresan M	3/4	"	90	0	81	0
16	Puraseed	3/4	"	87	0	77	0
17	Panogen	3/4	liquid	92	0	76	0
18	Chipcote	3/4	"	88	0	75	0
19	Setrete	3/4	"	75	0	75	0
20	Ceresan 100	3/4	"	89	0	76	0

At Lincoln soil conditions after planting apparently were not conducive to smut development. Infection in plants from untreated seed averaged only 3.7 percent. Twelve treatments eliminated smut and the remainder allowed only 0.1 percent or less (Table 1).

Seed of Rancher sorgo, which has persistent glumes, was mixed with spores of covered kernel smut, and separate portions were treated and planted as described for Combine-60 kafir. Data on germination, stand and control of smut are presented in Table 2.

None of the fungicides caused any marked reduction in emergence, and several seemed to cause some improvement. At Beltsville, where the checks averaged 37 percent infection, smut was eliminated by Phygon, Spergon, and the seven mercurials. Three other nonmercurials reduced infection to 6 percent.

At Lincoln, where infection in the checks averaged only 7.4 percent, the mercurials and seven of the nonmercurials gave complete control of covered smut. Infection percentages in the four other treatments ranged from 0.2 to 2.8 percent.

CROPS RESEARCH DIVISION, AGRICULTURAL RESEARCH SERVICE, UNITED STATES DEPARTMENT OF AGRICULTURE AND NEBRASKA AGRICULTURAL EXPERIMENT STATION, LINCOLN

A CURVULARIA LEAF SPOT OF ALYCE CLOVER

Stanley A. Ostazeski[1]

Summary

A leaf spot of Alyce clover caused by Curvularia maculans is herein described. The fungus caused symptoms similar to those formerly attributed to potash deficiency. The fungus grew and sporulated well on both V-8 juice agar and enriched potato-dextrose agar. Spores germinated on agar, but poorly in tap water, distilled water, or a 2 percent sucrose solution.

Alyce clover (Alysicarpus vaginalis (L.) DC.) is used as a late-summer forage crop in Florida. Good stands and yields have been obtained when it is planted on virgin land, but many crop failures have been recorded. These failures have been attributed to mineral deficiencies and root-knot nematodes. During the summer of 1957, Alyce clover fields near Dunellon, Florida, were examined to determine the cause of reduced stands. Plants in these fields were severely stunted, and much leaf spotting and some extensive necrosis occurred along leaf margins. Mineral deficiencies were associated with these poor stands, but the leaf spotting did not appear to be a nutrient-deficiency symptom. In surveys conducted during the summer of 1958 the disease was found in all fields observed, on both seedling and mature stands. The disease proved to be of parasitic origin and additional studies were conducted to determine some characteristics of the pathogen.

SYMPTOMS

The leaf spots are somewhat irregular but mostly circular (Fig. 1). The margin may be restricted by the veins. The spot has a tan to light-brown center with a thin, dark-brown bor-

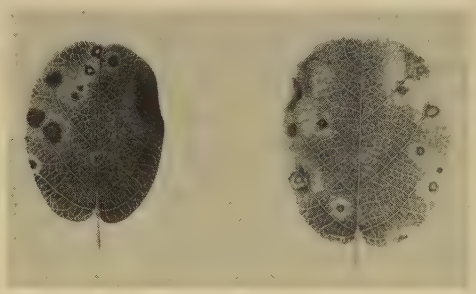

FIGURE 1. Leaf spots on Alyce clover caused by Curvularia maculans.

der, usually surrounded by a distinct, yellowish halo. The diameter varies from less than 1 mm to approximately 2 mm, but that of most spots is about 1 mm. Such symptoms on Alyce clover have been attributed to potash deficiency (1).

CAUSAL ORGANISM

A fungus identified as Curvularia maculans (Bancroft) Boed.[2] consistently sporulated on the surface of leaf lesions that had been rinsed in tap water and incubated in moist chambers at room temperature. When portions of diseased leaves were surface sterilized in 20 percent Clorox solution and plated on water agar or potato-dextrose agar, pure cultures of C. maculans were readily isolated.

Spore suspensions from 2-week-old cultures of C. maculans isolated from Alyce clover were sprayed on 2-month-old Alyce clover seedlings. These were immediately placed in a moist chamber. Check plants sprayed with tap water also were included in the test. After 3 days both

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[2] Thanks are extended to Dr. E. S. Luttrell for his identification of the fungus.

old and young leaves of the inoculated plants were uniformly spotted. Initially, the disease appeared as small chlorotic spots which soon enlarged and turned brown. Many of the young, heavily infected leaves were severely distorted. In general, symptoms were the same as on field-collected material except that the chlorotic halo was usually missing. C. maculans was reisolated from all inoculated plants sampled. The check plants remained disease-free.

Nelson (5) proved the pathogenicity of C. maculans on corn. Prior to his report the fungus was associated with diseases of rice (3, 6), pearl millet (4), pineapple (8), coconut (9) and pine (7). In these reports the pathogenicity of the fungus was undetermined, classed as being weakly pathogenic, or the organism was regarded as a secondary invader. This paper constitutes a second record of a disease caused by C. maculans.

Spores of C. maculans are typically 3-septate and slightly constricted at the terminal septum. The center cells are usually swollen and darker than the end cells. The spores are nearly barrel-shaped, and only a few are curved like typical Curvularia spores. Spores produced in Petri dish cultures on Difco potato-dextrose agar enriched with 5 grams of yeast extract per liter measured 20-39 x 12-19 microns and averaged 29.2 x 15.3 microns. They were more variable in color, size, and shape than those produced on infected host leaves incubated in moist chambers. Spores from host material measured 26-34 x 10-17 microns and averaged 30.4 x 13.3 microns. These measurements exceed the mean established by Boedijn (2) for C. maculans (24 x 14 microns). He recognized occurrence of the morphological strains of the species with differences too minute to warrant establishing them as distinct species.

Two isolates of C. maculans from Alyce clover were grown at 20°, 25°, 30°, and 35°C on V-8 juice agar and on enriched potato-dextrose agar. Radial growth was slightly better on the V-8 juice agar. The fungus grew best at 30° and poorest at 20°. Growth was about equal at 25° and 35°. Sporulation was profuse on both media at all four temperatures.

Spores germinated poorly in distilled water, tap water, or 2 percent sucrose solution. They germinated readily on water agar and potato-dextrose agar. After 5 hours the percentages of spores germinated were highest at 30°C, lowest at 20°, and about equal at 25° and 35°. After 8 hours germination had progressed so far that no temperature differences could be detected.

DISCUSSION

It is unlikely that the disease herein described is new in Florida. The brief description of potash-deficiency symptoms on Alyce clover by Blaser et al. (1) indicates that such symptoms resemble those caused by C. maculans. It is possible that the leaf spotting caused by the fungus has been mistaken for potash deficiency or that potash deficiency increases susceptibility to the disease.

Detailed studies are in progress concerning the pathogenicity and cultural characters of C. maculans isolates from different hosts and geographic areas.

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CROPS RESEARCH DIVISION, AGRICULTRUAL RESEARCH SERVICE, UNITED STATES DEPARTMENT OF AGRICULTURE, AND FLORIDA AGRICULTURAL EXPERIMENT STATION COOPERATING

COLLETOTRICHUM ANTHRACNOSE OF ALFALFA IN NEW YORK

D. A. Roberts, R. E. Ford, C. H. Ward, and D. T. Smith[1]

An epiphytotic of anthracnose, caused by Colletotrichum trifolii Bain & Essary, developed during the summer of 1958 in several fields of alfalfa near New Berlin, New York. In one first-year field of DuPuits alfalfa, about half of the plants were diseased and approximately half of the diseased plants had severe crown rot. Badly affected plants had wilted, bleached foliage and brittle stems, which could be broken off easily. Rotting tissues in the crowns and bases of stems were bluish black. Less severely affected plants had elliptical stem and petiole lesions with light brown centers and dark brown margins. Lesions sometimes coalesced and girdled stems. Symptoms observed on DuPuits alfalfa were the same as those described earlier for Colletotrichum anthracnose of alfalfa (2, 4). On Narragansett alfalfa attacked stems often were girdled by single lesions, but in three fields of this variety a lower incidence of disease and less crown rot were observed than in two nearby fields of DuPuits alfalfa.

The setose acervuli observed in stem lesions on both varieties of alfalfa bore straight, hyaline, nonseptate spores that were rounded at the ends and that measured about 4μ x 12μ. On the basis of symptoms and signs, the causal organism is thought to be C. trifolii. Fusarium spp. commonly occurred in stems attacked by C. trifolii, and when diseased tissues were plated on PDA, C. trifolii usually was overrun by the Fusaria. C. trifolii was isolated and obtained in pure culture by means of the dilution plate technique.

Anthracnose may cause extensive losses in alfalfa fields (1, 2), particularly in areas where Colletotrichum anthracnose of red clover is severe. The disease was observed on alfalfa in New York in 1907 (4), but no later reports of its occurrence there have been found. Probably it occurs rarely in the northern United States and in southern Canada; certainly its epiphytotic occurrence in a locality in New York is unusual.

Anthracnose occurred in all parts of the five fields inspected. Clover had not been planted for at least 6 years in the field where the disease was most severe, and probably neither the soil nor plant debris in the soil was the source of inoculum for the primary cycle. Red clover seedlings can be infected by C. trifolii from seed infested experimentally (3), and there is presumptive evidence that infested seed may be the source of inoculum for red clover under field conditions (2). Possibly infested seed was the source of inoculum for the disease outbreak reported here.

Although moisture requirements for anthracnose development probably were met during the unusually wet summer of 1958 in New York, daily mean temperatures near the area of disease outbreak averaged 5° to 10° below the 25° to 28° C optimum temperature reported for disease development in red clover (2). Possibly the strain of C. trifolii responsible for the epiphytotic reported here grows best at a temperature below 25°, and it could be that the disease it causes, particularly in DuPuits alfalfa, develops most rapidly at a temperature lower than 25° C.

The occurrence of alfalfa anthracnose gives little cause for alarm unless the variety DuPuits, which is being grown more extensively than before in the Northeast, is more susceptible than other varieties, or unless the optimum temperature for development of the disease caused by the New York strain of C. trifolii is lower than that for the development of the disease caused by other strains of the fungus.

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ABNORMALITY OF PEANUT SEEDLINGS CAUSED BY SEED INJURY

Norman C. Teter and Lawrence I. Miller[1]

Summary

Injury of the radicle tip and the orientation of peanut seed planted in soil have been demonstrated to influence hypocotyl curvature, rate of plant development, and production of multiple and fasciated primary roots. Hypocotyl curvatures and root malformations formed in the seedling stage are apparent in the plant at maturity. A mature, symmetrically formed peanut seed with a protruding radicle tip planted 1 1/4 inches deep developed into a symmetrical plant. A similar seed with the radicle tip injured a distance of 0.8 to 1.3 millimeters and planted at the same depth developed much more slowly and produced a seedling with a curled hypocotyl, and with multiple and fasciated primary roots.

Seedlings from both sound and mechanically injured seeds planted with the radicle down developed faster and had fewer root abnormalities than seedlings from seed planted with the radicle up, the hilum down, or the hilum sideways. Planting the seed with the hilum sideways resulted in more hypocotyl curvature of the seedling than when the seed was planted with the hilum up.

Germination of seed is not affected by mechanical injury to the radicle tip of the seed, but stands are impaired because of the slower emergence rate. Smaller kernels are occasionally produced on plants from seed with a mechanically injured radicle. Otherwise yield and commercial grade of peanuts from individual plants are not affected. However, if meaningful measurements of early plant development are to be made, it is important that injury to the seed radicle be prevented and that the seed be oriented uniformly in the soil when it is planted.

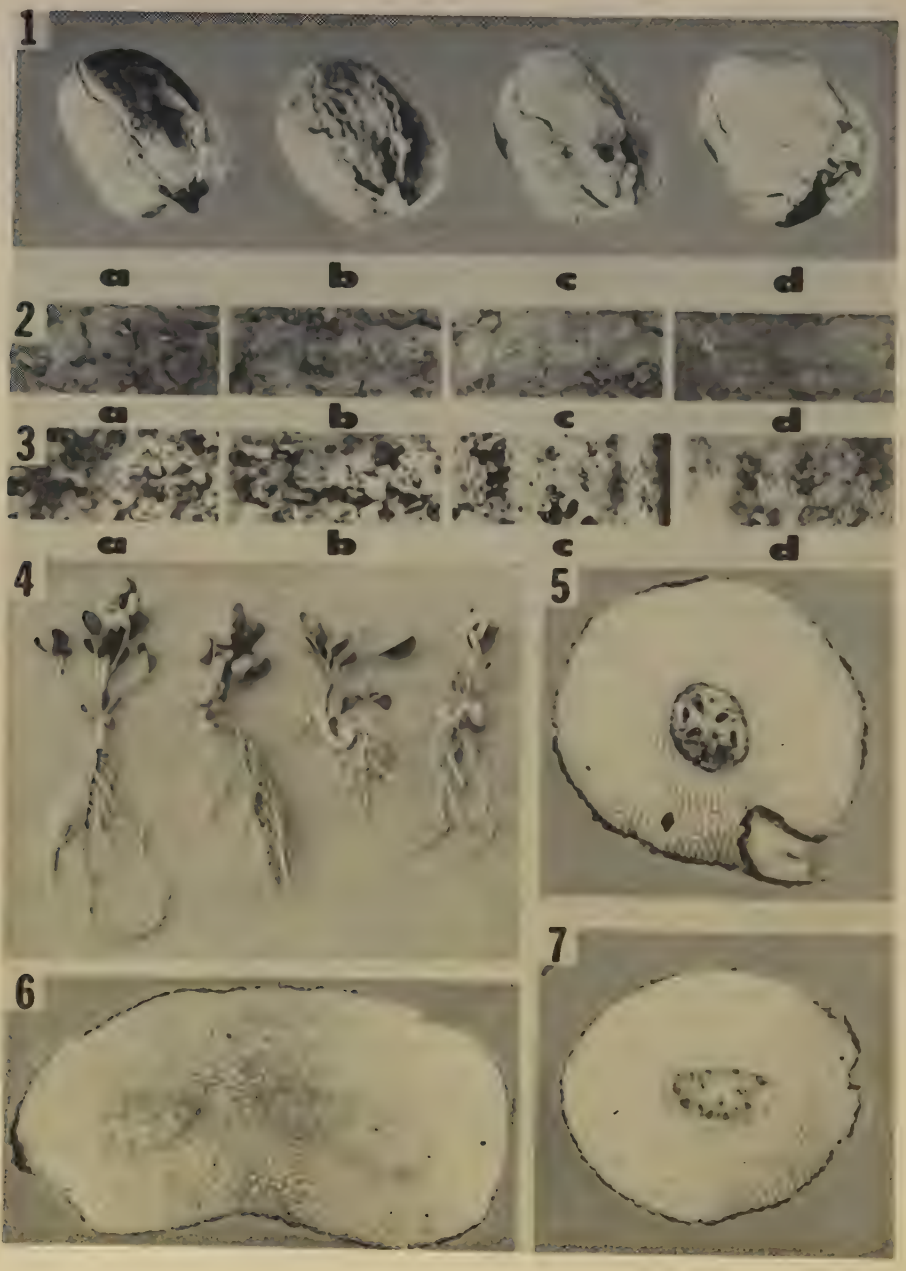
INTRODUCTION

Several workers have observed curvature of the hypocotyl of peanut seedlings, but curvature has apparently not been previously associated with radicle injury. Toole (8), in reporting the results of germination tests of seeds from experimental plots at Holland, Virginia stated that 14 percent of the seedlings from one lot of Virginia peanut seed tested in 1953 had very curled hypocotyls. Blackstone et al. (2) pointed out that germination is reduced when seeds are mechanically injured. Moore (5) states that although germination of mechanically injured seeds may not be significantly lowered, the emergence and early development of the seedlings are retarded. Darwin (3) in 1881 showed that injuries to the root apex caused curvature of the root. Preliminary tests in Virginia showed that there is an association between radicle injury and hypocotyl curling, and between radicle injury and development of the plant. A report of this study was presented by Teter and Miller in 1957 (7)

METHODS

A uniform seed selection of the SMJR Jumbo Runner variety peanut was used for the experiments in 1955 and 1956. Test lots were hand-shelled and only mature kernels with symmetrically formed and protruded radicle tips were selected for study. Studies were made of three treatments: full-cut, half-cut, and crushed, involving 0.8 to 1.3 millimeters of the radicle tip of the seed, to determine their effect on plant development. A sharp razor blade was used to sever the entire tip for the full-cut, and to make two right angle incisions to remove one-half of the tip for the half-cut. One thousand eight hundred grams of force applied with a slight rotation was used to obtain seed with a crushed radicle tip. Seed injuries are shown in Figure 1.

[1] The authors wish to acknowledge the technical assistance rendered by Robert L. Givens, Dorothy J. Rawls, and Betty J. Gray.



FIGURES 1 - 7. 1--Peanut seed injury treatments: A-check, B-full-cut, C-half-cut, and D-crushed. 2--Seedling emergence 7 days after planting: A-check, B-full-cut, C-half-cut, and D-crushed. 3--Seedling emergence 9 days after planting: A-check, B-full-cut, C-half-cut, and D-crushed. 4--Seedlings 14 days after planting, left to right: check, full-cut, half-cut, and crushed. 5--Transection, 1 1/2 cm from the root cap, of a normal primary root of a 14-day-old seedling. 6--Transection of a fused primary root of a 14-day-old seedling. 7--Transection of a cleft primary root of a 14-day-old seedling.

Experiments in the Greenhouse

Growth characteristics of injured and uninjured seed were compared. The test seeds were planted in wooden flats which were 5 1/2 inches deep and 25 inches square. Thiram-treated (3 ounces of 50 percent tetramethylthiuram disulfide per 100 pounds of shelled seed) seed were planted in check-rows 2 1/2 inches apart and 1 1/4 inches deep in a mixture of equal parts of sand and Woodstown loamy fine sand soil. The Virginia Extension Service (6) found an equivalent of 6 acre-inches of sand-soil mix contained the following: CaO, 373; MgO, 52; P_2O_5, 152; and K_2O, 104, in pounds. The pH was 6.0 and the organic matter analysis was 0.4 percent. In the preliminary tests all seeds were planted on their side. But in later tests records were made of seedling development from injured and uninjured seeds planted in four different positions: 1) radicle tip down, 2) radicle tip up, 3) hilum up and intercotyledonary plane vertical, and 4) with the hilum sideways and the intercotyledonary plane horizontal.

Tests were made to determine whether the thiram (3 ounces per 100 pounds of shelled seed) seed treatment or the organisms present in the sand-soil mixture influenced the abnormal seedling development of mechanically injured seed. Full-cut, half-cut, crushed, and check seed lots with and without thiram treatments were planted with the hilum up, and crushed seeds were planted with the hilum up in steam sterilized soil, methyl bromide-treated (3 pounds per 100 square feet) soil, and in nonsterile soil. The tests were repeated using planting flats that had been soaked in 5 percent formaldehyde for 1 hour and aerated 1 day before use.

Observations were made in the greenhouse on germination, rate of emergence, growth of leaves, curvature of hypocotyl, and on root malformations. Emergence was recorded photographically for each seed treatment at successive intervals. Ten days after the seed were planted, development of the individual plants was compared by counting the expanded leaves and by grading the size of the expanded leaflets. An arbitrary scale of 0 through 4 was used to rate the size of the leaflet according to surface area, with the upper maximum of 2.5 square centimeters. The degree of hypocotyl curvature was recorded as the angle between the negative directional growth of the central stem and the directional growth of the primary root or roots at the root collar portion of the hypocotyl. Primary roots were graded as single or multiple. Multiple primary roots were further graded as either fused or cleft, depending upon whether they were joined or separated.

To determine whether multiple roots should be classified as primary or secondary, a microscopic study was made of cell organization within the roots. Formalin-aceto-alcohol was used for fixation of the root samples. The tissue was dehydrated in ethanol and embedded in paraffin. Transections were cut 20 to 25 microns thick. A pararosanilin and anilin blue combination stain (4) was used satisfactorily in the study of the vascular elements of the roots. Sections were made from 0.5 to 1 centimeter from the apical root cap of 14-day-old seedlings.

Experiments in the Field

Emergence, incidence of bloom, root malformations at maturity, and the yield and commercial grade of fruit were recorded from tests in the field. In 1955 and 1956 the tests in the field were located at Holland, Virginia on Woodstown loamy fine sand soil. Chemical components, in pounds per acre, of the top 6 inches of the soil were: CaO, 445; MgO, 86; P_2O_5, 394; K_2O, 278. The pH was 6.0 and the organic matter content was 1.4 percent. A 2-year rotation of corn and peanuts had been followed for the previous 10 years in the test area. All agronomic practices prescribed by the Virginia Extension Service, except the use of herbicides for weed control, were followed in the field culture.

The treatments were arranged in a randomized block design and each plot consisted of one row 50 feet long. The rows were spaced 3 feet apart, with seeds placed by hand 6 inches apart in the drill row. The treatments were replicated four times in 1955 and six times in 1956. No attempt was made to orient the seed in the soil. To measure the yield per plant from the different treatments, all plots were thinned to a uniform stand when emergence was completed.

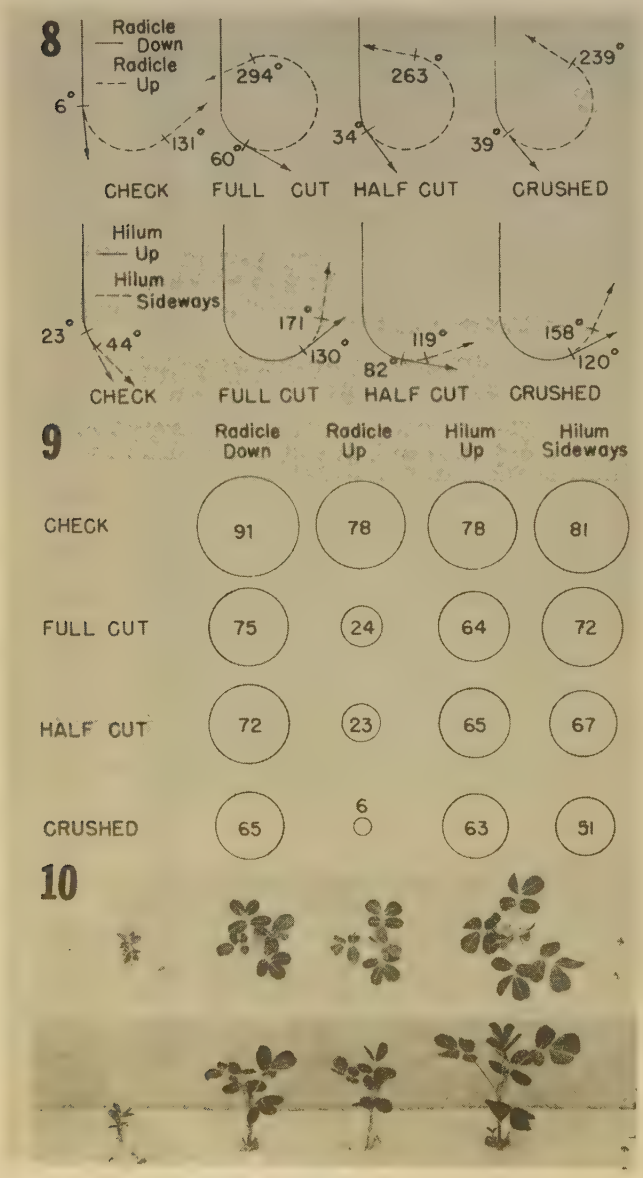
In 1955 the peanuts in the test area were planted May 25 and dug October 19. In 1956 the peanuts were planted May 16 and dug October 5. After the peanuts were dug they were field-cured, picked by hand, weighed, and then graded according to instructions issued by the Federal-State Inspection Service of 1952 (1).

RESULTS

Experiments in the Greenhouse

Germination of both injured and uninjured seed was excellent. However, the rate of emergence and early growth of the seedlings from injured seed was greatly retarded. The cut seed, both full and half, were not as drastically retarded as the crushed seed. Figures 2 and 3 show the stages of emergence 7 days and 9 days after planting the seed. Many of the check seedlings had developed four leaves while the seedlings from crushed seed were just emerging.

The measured effects of seed injury and seed orientation on the growth of 9-day-old peanut seedlings are both tabulated in Table 1. The foliar growth 10 days after planting is presented graphically in Figure 9. The size of the circle in the graph is proportional in area to the average leaflet size of the seedling, and the number within the circle shows the percentage of leaves expanded out of a maximum of four leaves per plant. In 10 days after planting, the check seed planted with the radicle down produced seedlings with an average of 3.6 leaves expanded and an average leaflet surface of 32 square centimeters, as compared with crushed seed planted with the radicle up which had 0.2 leaves expanded and a leaflet surface of 1 square centimeter. The foliar development of 14-day-old seedlings is presented in Figure 10.



FIGURES 8 - 10. 8 -- Effect of seed injury and seed orientation on the curvature of the hypocotyl. The radial dash across the curve symbolizes the root collar between the hypocotyl and the primary root. The length of the curve has no relationship to the length of the hypocotyl.
9 -- Foliar development of 10-day-old seedlings. The area of the circle is proportioned to the surface area of the average leaflet and the number in the circle is the percentage of a maximum of four expanded leaves.
10 -- Top and elevation views of 17-day-old seedlings, left to right: crushed, full-cut, half-cut, and check.

Table 1. Number of plants with two leaves expanded 9 days after planting 25 seed.

Radicle tip injury	Seed orientation in the soil			
	Radicle down	Radicle up	Hilum up	Hilum sideways
None	25	20	25	25
Full-cut	18	1	6	12
Half-cut	20	1	12	10
Crushed	11	0	10	6

Table 2. Oven-dry weight in grams of the central stem of peanut seedlings 14 days after planting.

Radicle tip injury	Seed orientation				Average
	Radicle down	Radicle up	Hilum up	Hilum sideways	
None	0.28[a]	0.26	0.31	0.30	0.28
Full-cut	0.27	0.21	0.28	0.29	0.25
Half-cut	0.28	0.18	0.30	0.30	0.27
Crushed	0.23	0.13	0.27	0.23	0.21
Average	0.27	0.20	0.29	0.28	

[a]Each value is an average of 25 readings.

Table 3. Percentage of plants with multiple (fused or cleft) primary roots when the hilum was positioned sideways[a]

Radicle tip injury	Fourteen day-old seedlings (greenhouse)[b]	Mature plants (field)[c]
None	0	3
Full-cut	40	44
Half-cut	56	60
Crushed	64	70

[a]All treatment differences were highly significant for both the seedlings and mature plants

[b]Average of 100 readings in 1955 and of 180 readings in 1956.

[c]Average of four replicates (35 readings per replicate) in 1955 and six replicates (30 readings per replicate) in 1956.

The oven-dry weight of the central stems, severed just above the cotyledonary attachment, 14 days after the seed was planted is listed in Table 2. The weights do not include the lateral branches which had developed on some plants at the time the measurements were made. Although the central stem weights were less for crushed seed and seed oriented with the radicle up, the differences in dry matter content from the different treatments were not as great as might be expected from visual inspection.

Typical seedlings of injured and uninjured seed lots are shown in Figure 4. Curvature of the hypocotyl was related to both the injury of seed and the seed orientation as shown graphically in Figure 8. Darwin (3) noted that radicles are always geotropic when they first protrude from the seed. There was no correlation between seed injury, orientation of seed at planting and length of the hypocotyl. The number of multiple roots produced is not necessarily greatest when hypocotyl curvature is greatest. Full-cut seed developed seedlings with the greatest hypocotyl curvature, but crushed seed produced more multiple roots as may be seen from the data presented in Table 3.

Arrangement of the vascular systems within normal primary roots (9) was quite different from the arrangement in roots classed as cleft primaries and fused primaries. A typical transection of each of these classifications is shown in Figures 5, 6, and 7. The normal root had a circular stele and a tetrarch vascular pattern, while cleft roots were unsymmetrical, with three prominent arches. Sometimes the fourth arch existed in a rudimentary fashion. The fused roots have two or more fasciated vascular cylinders and contain a variable number and arrangement of the xylary differention loci. Normal diarch secondary roots were, however, produced from cleft and fused primary roots.

The treatment of flats with formaldehyde may result in greater curvature of the hypocotyl, even though the flats are aerated for 1 day after treatment. However, with the techniques employed in these experiments, it was not possible to show that either the thiram seed treatment or the steam or methyl bromide soil treatments had any effect on hypocotyl curvature, root malformations, or rate of seedling development.

Experiments in the Field

The rate of emergence of crushed seed was significantly (0.01) less than that of full-cut and half-cut seed, and the emergence rate of full-cut and half-cut seed was significantly (0.01) less than that of the uninjured seed. There was no difference between the final emergence count of the uninjured peanuts and that of seed with the two types of cut injuries, but the final emergence count of crushed seed was significantly (0.05) lower. In 1956 the percentage of seed that emerged 15 days after planting was: check, 86.5; full-cut, 73.2; half-cut, 73.2; and crushed, 63.5. Forty-three days after planting the percentage emergence readings for the same plots were: check, 88.8; full-cut, 88.8; half-cut, 87.2; and crushed, 81.5.

The plants from uninjured seed had significantly (0.05) more blooms per plant 40 days after planting than the plants from injured seed. The bloom counts were: check, 1.0; full-cut, 0.2; half-cut, 0.4; and crushed, 0.1. Forty-seven days after planting, the number of blooms per plant were: check, 9.9; full-cut, 6.3; half-cut, 6.9; and crushed, 4.9. There were significantly (0.05) fewer blooms on plants from injured seed than from uninjured seed and significantly (0.05) fewer blooms on plants from crushed seed than from cut seed.

Hypocotyl curvatures of 55° (check), 122° (full-cut), 105° (half-cut), and 140° (crushed) of mature plants from the field correspond to the average hypocotyl curvatures of 34° (check), 150° (full-cut), 100° (half-cut) and 139° (crushed) of seedlings grown in the greenhouse from seed planted either with the hilum up or hilum sideways (Figure 8).

Plants from half-cut seed had significantly (0.05) fewer fused primary roots than did those from full-cut and crushed seed. There was no statistical difference between the number of cleft roots on plants from full-cut and half-cut seed, but plants from either type of cut injury had significantly (0.05) fewer cleft roots than those developing from crushed seed.

With one exception, the yield per plant and grade of fruit from the various treatments was not significantly different. In 1955 there were significantly (0.05) fewer Extra Large kernels (seed retained by a screen with slots 22/64-inch in width) produced by plants grown from crushed seed than from plants grown from the other three lots of seed.

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AGRICULTURAL ENGINEERING RESEARCH DIVISION, UNITED STATES DEPARTMENT OF AGRICULTURE, AND VIRGINIA AGRICULTURAL EXPERIMENT STATION

STUB-LEAF OF PEANUT (ARACHIS HYPOGAEA)

Carl Hartley[1] and W. K. Bailey[2]

In an earlier paper[3] pale dwarf, a juvenile disease of peanut in Java was described. Another quite different type of juvenile disease, which will be referred to as stub-leaf, was also encountered there in collaboration with Dr. M. B. Schol-Schwarz. The typical condition is shown in Figure 1.

FIGURE 1. Java Holle peanut plant showing typical stub-leaf condition.

As in pale dwarf, roots and hypocotyls were usually well developed and the disorder was most frequent in seedlings from old seed. Stub-leaf differed from pale dwarf in the following ways:

(1) The first leaves had petioles but no leaflets; on some of them even the petioles were reduced or lacking, but on most of them the stipules were of normal size.
(2) Petioles were no more slender than normal, and usually attained normal length.
(3) Affected leaflets which were not entirely suppressed lacked the slender shape seen in those affected with pale dwarf, but showed characteristic crinkling. Instead of being pale, they were often darker green than normal (Figure 2).
(4) Stub-leaf was most prevalent in plants germinated during wet, relatively cool weather; the two plantings that showed the most stub-leaf were entirely free from pale dwarf.
(5) Recovery of the affected plants was abrupt instead of gradual, and the effect on the ultimate development of the plant was less than that of pale dwarf. The third or fourth leaf (the fifth in Figure 3) and all subsequent leaves on the main stem and the first pair of leaves on the side stems were ordinarily normal.
(6) In extreme cases, despite normal root and hypocotyl, no epicotyl appeared and decay finally developed in the pith of the hypocotyl and upper root.

The field planting in which stub-leaf was most in evidence included 39 lots of seed from various parts of Indonesia. Most of these had been in storage beyond the usual time. The incidence of typical stub-leaf (one or more leaflets entirely absent) ranged from 1 to 78 percent, the highest figure being for a lot from Djokjakarta, Java, which in a previous greenhouse test had produced 85 percent of plants with stub-leaf. In this field planting germination was generally poor, with emergence below 75 percent in more than half of the lots. More stub-leaf was found in the lots with the lower emergence percentages, but this inverse correlation between emergence and stub-leaf incidence was not as pronounced as it had been in the data cited on pale dwarf.

All three types of peanut grown in Indonesia showed the stub-leaf condition. In the worst planting stub-leaf affected 23 percent of the important Holle (bunch type), 18 percent of the

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[3] Hartley, Carl. 1927. Pale dwarf disease of peanut (Arachis hypogaea) Phytopathology 17: 217-225.

FIGURE 2. The dark green crinkled-leaf condition regarded as mild stub-leaf.

FIGURE 3. Stub condition in first three leaves and part of the fourth.

FIGURE 4. Cross section of Virginia Bunch peanut seeds showing dark brown plumule (left), plumule with brown tips (center), and normal plumule (right).

Broel (Spanish type), and 14 percent of the Tjina (runner). Leaf crinkle without definite stub-leaf was present in addition on half of the Holle and Broel plants and a third of the Tjina in this planting, leaving less than half of the seedlings entirely normal in appearance. The crinkle condition was frequent in seedlings of "Boschneger Pinda" and "3-seeded Pinda" grown from seed just received from Dutch Guiana; in the latter variety typical stub-leaf also occurred. In most plantings stub-leaf was much less frequent than in this one, and in no other did it have any obvious importance in reducing yield.

In the United States both stub-leaf and crinkle have been noted on Virginia type peanuts. Observations by one of the writers in Virginia and Georgia, especially on the large seeded varieties Virginia Bunch 46-2 and Georgia 119-120, show seed abnormalities that appear related to the subnormal shoot development. In some lots of seed, plumules otherwise normal in appearance vary in color through various shades from light yellowish tan to dark brown (Figure 4). The early stages of germination and growth were observed directly in divided seed during germination in moist chambers. The lightest brown plumules became green during germination and grew normally. Plants from the seed with the darkest plumules developed stub-leaf or crinkled leaflets; on part of these some of the petioles were stunted or failed to grow, and in extreme cases there was no epicotyl growth even though root and hypocotyl were normal.

Seed planted in soil after the presence of browned plumules has been ascertained by clipping off the distal tip of the seed showed relatively poor percentage of emergence and all degrees of epicotyl deficiency, including seedlings with normal hypocotyl and root whose cotyledons emerged without epicotyls. The seedlings with the less extreme leaf abnormalities were kept under observation longer than in Java, and though they subsequently produced normal leaves many of them were low in top vigor during the first few weeks. The side shoots were affected more than had been noted in Java, and it is probable that the effect on yield is somewhat larger than was supposed from the observations made there. The condition that causes the browning of the plumules apparently continues for some time to affect the ability of the top to utilize the food in the cotyledons.

Seed with brown plumules were most commonly noted from parent plants that had been subjected to severe drouth after they were fully grown. In extreme cases such seed show shrunken spots on the outer surface. Less severely affected seed fail to show quite normal smoothness and color. The seed with brown plumules usually have externally plump cotyledons, but often are concave, so that the void between the cotyledons is unusually large.

At first it was supposed that the shoot deficiencies might be due to the injury to the plumule of weak or old seed by fungi during the first stage of germination. In the American seed examined at different stages of sprouting, fungi occasionally destroyed the embryo, but usually started on the cotyledons or the root, and the plumule was the last part to be attacked. It is also worth noting that the seedling abnormalities due to mechanical injury to the embryo[4] do not include the stub-leaf or the type of crinkle associated with it. Only a very few Virginia seed subjected to various drying treatments by Mr. Normal C. Teter and included in germination tests by Dr. and Mrs. E. H. Toole showed stub-leaf.

The inference drawn from the incomplete data available is that the stub-leaf defects described in the foregoing from Java and the United States are identical and have an environmental cause rather than microorganisms or genetic factors. Withdrawal of water from the plumule by drouth occurring in the last stages of maturation of the seed is regarded as a possible cause.

CROPS RESEARCH DIVISION, AGRICULTURAL RESEARCH SERVICE,
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BOTRYTIS LEAF BLIGHT OF RICINUS COMMUNIS L.

R. G. Orellana[1]

Summary

An outbreak of Botrytis leaf blight of castorbeans at the Florida Experiment Station in 1958 is reported. Inoculation of five castorbean strains with Botrytis showed that they varied in susceptibility and suggested that leaf reaction to the fungus may be used as an aid in the evaluation of disease resistance.

Botrytis leaf blight of castorbeans (Ricinus communis L.) is incited by Botryotinia ricini (Godfrey) Whet. usually reported in the conidial stage as Botrytis sp. This fungus also causes the destructive gray mold of the capsules and discourages the commercial production of this crop where the disease is endemic. Losses resulting from the attack of castorbean capsules are often extremely high.

Although Godfrey[2] described the fungus as initiating its infective cycle on the leaves, inflorescences and stems, he stated that infections of the leaves and stems do not occur readily. This paper reports an outbreak of Botrytis leaf blight which developed simultaneously with gray mold and continued after it had attacked the flowers and capsules on experimental castorbean plantings at the Florida Experiment Station in Gainesville in 1958. Results of some inoculations with Botrytis are also described.

The first molded racemes at the Gainesville plantings were observed late in July. Subsequently their number increased and did not decline until late August. Botrytis leaf blight, on the other hand, prevailed until November. Precipitation and relative humidity dropped abruptly from August to September and air temperature from September to October at Gainesville. In southern Florida Botrytis leaf blight and capsule mold were both found to occur during November on volunteer castorbean plants.

LEAF BLIGHT SYMPTOMS

Godfrey observed that Botrytis leaf blight of castorbeans is initiated by infected inflorescence-tissue fragments which fall upon leaves[2]. Undoubtedly spore clouds, which readily form when molded inflorescences are disturbed, contribute to the spread of the disease. Insects and rain splash and run-off have also been mentioned as playing roles in the dissemination of the pathogen.

Under field conditions blight lesions (Fig. 1) are circular or subcircular and vary in size from 4 to 5 mm in diameter to lesions affecting large portions of the leaf. The lesions increase in area by coalescence of smaller lesions. Blight develops either marginally or apically and near or around the base of the leaf where dew or rain usually collects. In the latter case the upper portion of the petiole develops rot and collapses.

The lesions are pale brown and under high humidity become covered with a delicate web of Botrytis mycelium. Generally the blighted area does not fall out. If dry conditions prevail, it may remain part of the leaf for some time. Large blighted areas on the leaves show zonation which marks the periodic advances of the disease.

INOCULATION EXPERIMENTS

Four Botrytis isolates were used for inoculating seedlings in the greenhouse. Isolates 2-C and 37-C from molded castorbean capsules were collected at the Plant Industry Station of the United States Department of Agriculture, Beltsville, Maryland, and the Florida Experiment Station, Gainesville, Florida, respectively, and 38-C and 46-C were obtained from castorbean leaves with Botrytis blight collected at the latter location. The Beltsville isolate 2-C differed from the three Gainesville isolates in cultural characteristics.

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[1] Plant Pathologist, Crops Research Division, Agricultural Research Service, United States Department of Agriculture, and the Department of Plant Pathology, University of Florida at Gainesville.

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FIGURE 1. Symptoms of Botrytis leaf blight from natural infection of castorbeans in the field at the Florida Experiment Station, Gainesville, Florida.

Inocula were increased in castorbean leaf decoction, and the mycelial mats were triturated in water to make a suspension. Inoculation was effected by spraying the leaves with this suspension.

The pathogenicity of each of the isolates was tested by spraying suspensions of them on plants in the two- and four-leaf stages in the greenhouse. These plants were kept under conditions of high humidity and developed blight in 48 to 72 hours after inoculation. The fungus was re-isolated and identified as Botrytis.

Symptoms induced in the greenhouse were similar to those occurring naturally under field conditions, but in the greenhouse zonation of the blighted area was not always conspicuous. Frequently when the center of the leaf was blighted, the leaf blade folded like an umbrella, The petiole became infected, and the leaf fell. Scars left by the fallen blighted leaves became infected, and localized Botrytis cankers developed on the stem. Cankering did not result as readily on woody stems as on young succulent stems.

Inoculation by spraying Botrytis suspensions on 11 castorbean strains in their two-leaf stage, 8 days after emergence, caused infections that did not differ with the strain of castorbeans. All of the seedlings were eventually killed by the disease. When a new series of five castorbean strains 1-month-old were each sprayed with the suspension of each of the four Botrytis isolates, blight ranging from mild to severe developed within 72 hours. Since these five castorbean strains apparently varied in their susceptibility to the disease, leaf reaction to Botrytis may be used as an aid in the evaluation of disease resistance.

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CALCIUM DEFICIENCY IN COTTON SEEDLINGS[1]

Alfred B. Wiles[2]

Summary

The absence or unavailability of calcium for utilization by cotton seedlings produces symptoms ranging from collapse and death of the primary radicle, the terminal bud, and portions of the hypocotyl, to chlorosis and necrosis of the cotyledonary leaves and stunting of the young plants. Both temperature and the past history of the seed were found to be influencing factors, and preliminary results relating to these factors are reported. The symptoms of calcium deficiency closely resemble those which have been attributed entirely to soil-borne fungi. Adequate control of calcium deficiency was obtained when fuzzy or machine-delinted cotton seed were rolled in dry calcium sulfate prior to planting. Because of the possible role that this deficiency may play in the cotton seedling disease complex, further studies are in progress, particularly regarding temperature and seed deterioration.

INTRODUCTION

The need for calcium for proper growth and development of seedlings was noted in an early paper by True (7), and later by Albrecht (1). Sorokin and Sommer (6) described the effect of the absence of calcium cells and tissues of garden pea, and Kalra (3) gave a detailed account of this effect on tomato. Haynes and Robbins (2) found calcium to be essential to survival of tomato roots and reported that in the presence of only calcium and boron the roots maintained their normal function. The role of calcium in producing healthy radicles of cotton was described by Presley and Leonard (4). They did not, however, describe any further symptoms of this deficiency or indicate a possible relationship with the seedling disease complex. A recent report by Ranney and Bird (5) listed calcium chloride and calcium nitrate along with various other compounds as being effective in helping to maintain stands of cotton in which seedling diseases were present. The present studies were begun as an outgrowth of the difficulty experienced in growing healthy cotton seedlings in white quartz sand and tap water under relatively cool temperatures. The objective was to determine the reaction of these seedlings to the Verticillium wilt organism under optimum conditions for disease expression. When greenhouse temperatures were held at about 20° C a large portion of the young plants developed symptoms similar to those encountered in the field where seedling diseases occurred. Since no organism could be found associated with this condition in the greenhouse, it was suspected of being nutritional in nature.

SYMPTOMS ON COTTON

On the radicles of cotton seedlings symptoms vary from moderate browning of a portion of the tap root to complete breakdown (Figure 1). In some cases only the terminal portion of the tap root dies and lateral roots may subsequently be produced in the surviving area. When such seedlings survive they have a root system devoid of a primary root, resembling a condition in older plants known as "nub-root." The above symptom may be confused with a like condition in cotton seedlings, resulting from planting deteriorated seed rather than from calcium deficiency. Affected radicles have a brownish, translucent, water-soaked appearance suggestive of fungal attack. At the terminal bud there is usually complete collapse and death of the tissue. Plants often show collapse of a large portion of the hypocotyl. Yellowing and necrosis of the cotyledonary leaves occur less regularly than death of the terminal meristems and most often follow death of the terminal bud.

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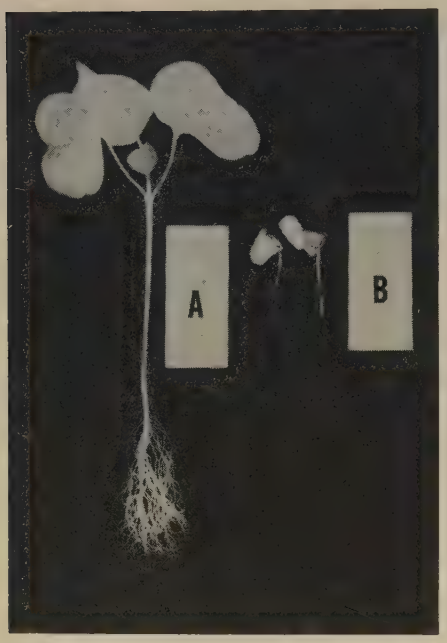

FIGURE 1. Calcium deficiency in cotton seedlings: A -- Plant grown from calcium sulfate-treated seed in white quartz sand watered with distilled water; B -- Plants grown under identical conditions except for omission of calcium sulfate. Note breakdown of primary radicle.

MATERIALS AND METHODS

The medium in which the plants were grown was white quartz sand in 2-gallon glazed crocks. Both tap and distilled water were used. The tap water had the following composition in parts per million: SiO_2, 8.0; Fe, 0.2; Al, 0.3; Ca, 8.0; Mg, 0.8; Na+K, 33.3; CO_3, 0.0; HCO_3, 102.5; SO_4, 1.9; NO_3, 0.0; Cl, 13.0; and F, 0.10. After the sand was placed in the crocks it was washed three times with tap water, and if distilled water was to be used the sand was washed a fourth time with distilled water. Two-year-old fuzzy cotton seed which had received no seed fungicides were used throughout. All seed were of the same strain and were harvested from the same field plot on the same date. For each test sufficient seed to plant 24 crocks at the rate of 15 seed per crock were rolled in dry calcium sulfate prior to planting. Another 24 crocks were planted to seed which had received no calcium sulfate. Half of the crocks in each of the series were watered with distilled water and half with tap water. These studies were conducted in the greenhouse during the early summer of 1958 when temperatures were high, and in controlled-temperature refrigerators with artificial lighting. The results of three separate tests are reported. These involved: 1) placing all crocks in the greenhouse at warm temperatures (24°C and above); 2) placing half of the crocks in the greenhouse at warm temperatures and the other half alternately in refrigerators at 20° for 16 hours and then in the greenhouse for 8 hours during the day; and 3) placing half of the crocks in the greenhouse and half in the refrigerator at 20° continuously for 15 days.

RESULTS

Results obtained from the first test were as follows: calcium deficiency symptoms appeared only in the six crocks of plants that received distilled water and no calcium sulfate. The plants that received the calcium sulfate emerged more rapidly and tended to be more vigorous; however, there were no deficiency symptoms in the plants receiving only tap water. Results obtained in the second test were identical with those obtained in the first test. Exposure to temperatures of 20°C for 16 hours and warm temperatures for 8 hours did not bring about calcium deficiency symptoms, except where the plants received only distilled water. In the third test, where half of the plants were exposed to a temperature of 20° for 15 days, over 75 percent of the plants in the crocks receiving only tap water and over 90 percent of those receiving only distilled water showed severe calcium deficiency symptoms. None of those receiving calcium sulfate showed deficiency symptoms.

Further studies are underway involving the use of calcium on deteriorated cotton seed,

since it has been found that such seed respond to calcium at both high and low temperatures.

DISCUSSION

In Mississippi seedling diseases cause losses of young cotton plants primarily in light, sandy soils during and following periods of low temperature. Since the seed are planted at an average depth of about 1 inch, it seems possible that a calcium-deficient condition could prevail in the restricted area around the developing plant for a sufficient time to produce the symptoms described. This does not imply that soil fungi do not attack cotton seedlings, but it is suggested that healthy, vigorous seedlings such as those obtained from the calcium sulfate-treated seed could better resist attack by such organisms. It is further suggested that some of the possible benefits attributed in the past to the use of certain materials as soil fungicides are actually related more closely to the calcium compounds used as diluents rather than to the fungicidal action of the material itself. Under field conditions the writer has repeatedly observed symptoms on cotton which appeared identical with those produced in the greenhouse.

While the results obtained where the low temperatures were involved are preliminary, they strongly suggest that low temperatures coupled with an insufficient supply of calcium are responsible for the difficulty encountered in the greenhouse testing program. Since these findings could have a bearing on the cotton seedling disease complex, it appears that further studies are warranted.

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MISSISSIPPI AGRICULTURAL EXPERIMENT STATION, AND CROPS RESEARCH DIVISION, AGRICULTURAL RESEARCH SERVICE, STATE COLLEGE, MISSISSIPPI

REDUCTION IN YIELD OF COTTON CAUSED BY DISEASES IN 1958

Compiled by the Cotton Disease Council, Committee on Disease Losses: Harlan E. Smith, Chairman; A. L. Smith, W. E. Cooper, Leonard Lett.

The accompanying tabulated summary of the 1958 Cotton Disease Loss Estimates is the seventh report submitted by the committee on cotton disease losses.

The summary was compiled as before, from 51 estimates received from cooperators in the States reporting. The accuracy of the estimates is highly reliable, since most of the same cooperators have contributed for the last 7 years and, as a consequence, their methods and techniques are becoming uniform. The committee feels that considerable credence can be placed in the report.

The committee wishes to acknowledge the help of all who contributed to the report and to solicit their further cooperation in making the cotton disease loss summary a continuing success.

The list of cooperators follows:

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Table 1. ESTIMATED REDUCTION IN 1958 COTTON YIELD AS A RESULT OF DISEASE DAMAGE.

Disease	Calif.	Ariz.	N. Mex.	Tex.	Okla.	Mo.	Ark.	La.	Miss.	Ala.	Ga.	S.C.	N.C.	Tenn.	Bales Lost	Percent Loss
1. FUSARIUM WILT *Fusarium vasinfectum*	--	--	--	0.07	0.20	5.00	2.00	2.50	1.65	1.00	1.75	1.00	1.00	1.75	101,821	.74
2. VERTICILLIUM WILT *Verticillium albo-atrum*	2.83	2.76	5.75	3.06	1.80	3.00	3.00	.20	1.10	tr	--	--	0.40	2.00	331,384	2.42
3. BACTERIAL BLIGHT *Xanthomonas malvacearum*	.33	tr	.37	7.31	5.20	7.50	1.50	0.30	.35	0.20	1.00	.10	1.00	0.75	468,026	3.42
4. ROOT ROT *Phymatotrichum omnivorum*	.33	3.01	tr	3.18	0.30	--	tr	tr	--	--	--	--	--	--	198,718	1.45
5. ANTHRACNOSE BOLL ROT *Glomerella gossypii*	--	--	--	--	--	1.00	--	tr	--	1.50	.50	.10	0.30	2.00	24,502	.18
6. SEEDLING DISEASES *Rhizoctonia*, etc.	2.50	2.11	1.75	3.00	0.60	1.00	1.75	3.00	2.50	3.00	3.50	1.20	3.00	2.00	343,859	2.58
7. ASCOCHYTA BLIGHT *Ascochyta gossypii*	--	--	--	0.04	--	.20	.25	tr	tr	tr	tr	.10	0.20	0.75	10,281	.07
8. BOLL ROTS *Rhizopus*, etc.	3.66	1.16	.50	0.92	2.00	1.00	5.00	3.80	7.00	3.50	1.75	.20	0.50	0.50	322,240	2.35
9. ROOT KNOT *Meloidogyne sp.*	1.00	1.59	1.75	0.24	0.10	5.00	tr	tr	.50	3.50	2.50	6.00	2.00	1.25	136,346	.91
10. OTHERS	tr	.26[a]	tr	0.12	--	1.00[b]	.40	tr	--	--	.50	.20	0.10	0.10	20,601	.15
TOTAL PERCENT LOSS	10.65	10.89	10.12	17.84	10.20	24.70	13.90	9.80	13.10	12.70	11.50	8.90	8.50	11.10		14.27
TOTAL BALES LOST	196,671	97,779	32,089	927,657	37,483	95,126	163,054	32,051	155,270	65,463	48,079	29,797	23,224	53,689	1,957,778	
YIELD IN THOUSAND BALES 1958[c]	1,650	800	285	4,250	330	290	1,010	295	1,030	450	360	305	250	430		

[a]Crazy top, rust (*P. cacabata*), Alternaria leaf spot and leaf crumple
[b]Cercospora leaf spot
[c]Nov. 1, 1958 BAE Estimate

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COTTON DISEASE COUNCIL, COMMITTEE ON DISEASE LOSSES

PUBLICATIONS RELATIVE TO VIRAL DISEASES OF PLANTS FROM 1900 to 1956

H. H. Thornberry[1]

Since the beginning of modern plant virology about 1900, the number of published scientific papers pertinent to virology in relation to the number on all plant diseases increased to about 30 percent by 1956 (estimates in Table 1 and the trend illustrated in Figure 1). If these estimates are indicative of the trend, the percentage of papers relative to virology should be 40 percent by 1974 and 50 percent by 1992. It is assumed that the number of publications is indicative of the amount of investigations.

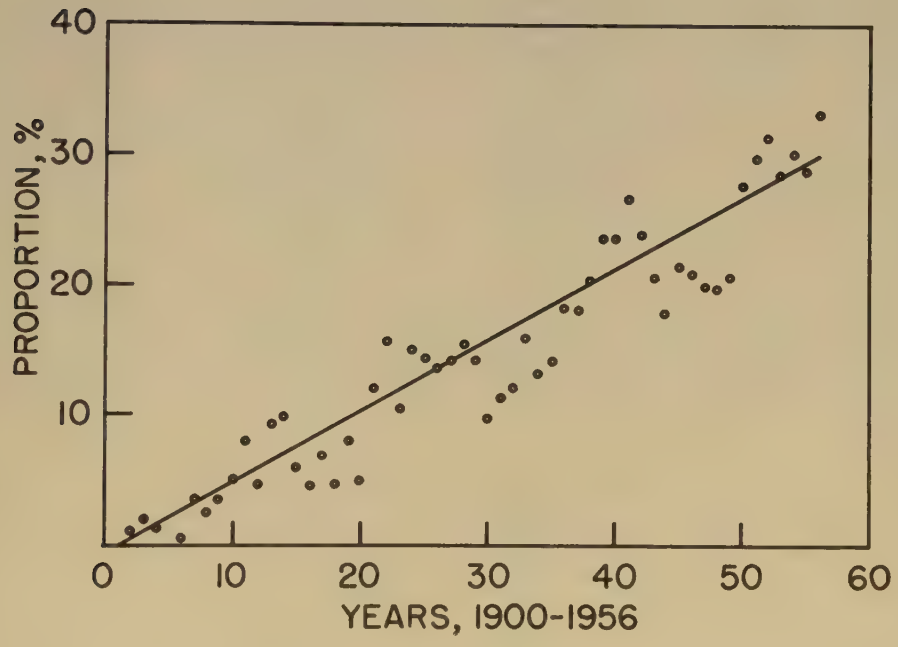

FIGURE 1. A graph showing the proportion, percent, of the number of abstracts pertinent to virology to those relative to all diseases for the period from 1900 to 1956 (Plotted data from column 4 of Table 1).

These data are published for what values they may have for inquisitive readers or planners of research programs.

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Table 1. Number of abstracts of scientific papers relative to all plant diseases and to viral diseases in Experiment Station Record from 1900 through 1946 and in Review of Applied Mycology from 1947 through 1956.

Year	Number of abstracts: All diseases (estimated[a])	Number of abstracts: Viral diseases (counted)	Abstracts on virology (percent)
Experiment Station Record:			
1900	2	2	100.0
1	0	0	0.0
2	325	3	0.9
3	235	5	2.1
4	245	3	1.2
1905	60	5	8.3
6	15	0	0.0
7	525	19	3.6
8	575	16	2.7
9	460	16	3.4
1910	555	28	5.0
1	350	28	8.0
2	475	23	4.8
3	560	53	9.4
4	525	42	9.9
1915	410	25	6.0
6	590	26	4.4
7	555	40	7.2
8	570	28	4.9
9	450	37	8.2
1920	560	28	5.0
1	695	85	12.2
2	615	97	15.7
3	680	72	10.5
4	625	94	15.0
1925	635	91	14.3
6	700	96	13.7
7	880	126	14.3
8	915	149	15.5
9	640	91	14.2
1930	550	54	9.8
1	630	72	11.4
2	765	93	12.1
3	630	102	16.1
4	800	106	13.2
1935	905	129	14.2
6	955	175	18.3
7	920	168	18.2
8	835	170	20.4
9	855	204	23.8

Table 1. (continued)

Year	Number of abstracts: All diseases (estimated[a])	Number of abstracts: Viral diseases (counted)	Abstracts on virology (percent)
1940	770	182	23.6
1	815	217	26.6
2	895	214	23.9
3	880	181	20.5
4	885	155	17.5
1945	660	143	21.6
6	760	150	19.7
Review of Applied Mycology:			
1947	1083	215	20.1
8	1113	243	19.9
9	1233	343	21.7
50	1203	359	27.9
1951	1203	379	29.9
2	1203	344	31.5
3	1323	397	28.6
4	1443	418	30.0
5	1410	491	28.9
1956	1744	580	33.2

[a]At 5.0 abstracts per page in Experiment Station Record and at 3.12 abstracts per page in Review of Applied Mycology, but with corrections for abstracts not pertinent to plant pathology (Correction factor 0.66).

ILLINOIS AGRICULTURAL EXPERIMENT STATION, URBANA

EXOCORTIS-LIKE SYMPTOMS ON UNBUDDED SEEDLINGS AND ROOTSTOCKS OF PONCIRUS TRIFOLIATA WITH SEEDLING-LINE TOPS, AND PROBABLE SPREAD OF EXOCORTIS IN A NURSERY

E. C. Calavan, R. K. Soost, and J. W. Cameron[1]

Summary

Subterranean symptoms resembling but not typical for those of exocortis have appeared on presumably uninoculated seedlings and rootstocks grown from seed of a similarly affected diploid trifoliate orange parent tree. Probable spread of exocortis within a nursery in the direction of water flow, is reported.

OBSERVATIONS

The recent discovery in many presumably uninoculated trees, including both seedlings and rootstocks of trifoliate orange, Poncirus trifoliata (L.) Raff., of bark symptoms resembling but not typical of those of exocortis (scaly butt) provided the basis for this report. Although minor variations in trunk and bark characters have been observed frequently in trifoliate orange, very few prior instances of associated severe bark deterioration have been noted except when exocortis has been present.

Cracking of the bark, accompanied by the death of outer portions of the bark below the soil level, was noted at Riverside, California in October 1958, on two 14-year-old seedling progeny from a diploid P. trifoliata seedling. Excavation of the lower trunk of the 25-year-old parent tree showed the same pattern of cracking found in the two daughter seedlings (Fig. 1). The cracks extended a depth of 1 to 3 mm into the bark and ranged from about 1 cm to more than 15 cm in length. Orientation was mostly longitudinal. Distances between cracks varied from about 1 cm to nearly 5 cm. Bark tissues between the cracks were dead to a considerable depth, usually 1 to 2 mm below the bark surface. In dry soil the dead bark between the cracks was very hard and adhered tightly to the living portion of the bark. Very few exfoliating areas of bark were found. The trunks of all three diploid seedling trees were markedly constricted below ground level (Fig. 1).

The 25-year-old diploid seedling was one of two trifoliate orange trees developed by H. B. Frost and used by him in 1944 as rootstock seed sources for a block of navel orange (Citrus sinensis (L.) Osbeck), mandarin (C. reticulata Blanco), and mandarin-hybrid trees propagated in 1946 and planted in 1949 at the University of California Citrus Experiment Station, Riverside. The other tree used in 1944 as a seed parent was a symptomless tetraploid seedling with greater vigor than the diploid. These two parent seedlings grew in the same nursery row and probably had a common seed parent. Diploid and tetraploid conditions for some of the progeny of the parent diploid and tetraploid trees, respectively, have been verified by chromosome counts (5).

Altogether, buds from 18 scion parents of 15 varieties were propagated on 57 diploid and on 54 tetraploid trifoliate orange seedling rootstocks.

In October 1958 the soil was removed to a depth of about 12 cm around the base of each tree. The trunks then were examined for bark cracks, bark shelling and constrictions. Data obtained are presented in Table 1. Forty-six of the 57 diploid rootstocks had underground bark cracks (Fig. 2) like those of the parent seedling. Twenty-three of these also were constricted (Fig. 2) below ground level. Trunks without cracks lacked abrupt constrictions. Five of the 46 diploid rootstocks with bark cracking had considerably more exfoliation of bark (Fig. 3) than the other 41. Exfoliation on these five rootstocks, although largely subterranean, is believed to be due to exocortis infection. Tops of four of these five trees were propagated from a Temple tangor (C. reticulata x C. sinensis) known to carry exocortis virus. The top of the fifth tree is Kinnow mandarin from a young-line parent. Exocortis virus from the Temple tangor also caused exfoliation on the four tetraploid trifoliate orange rootstocks on which the diseased scions were propagated (Fig. 3). One of each of the tetraploid rootstocks with Kinnow mandarin and Ruvel orange tops, respectively, developed severe exocortis symptoms but three other trees of each of these combinations showed no external evidence of exocortis.

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FIGURE 1. Trunks of uninoculated trifoliate orange seedlings. Left -- 25-year-old Frost diploid parent tree with deep bark cracks, tight dead outer bark and constricted zone, all below ground level. Center -- 14-year-old Frost diploid, a first-generation seedling of the tree at the left. Right -- Normal 14-year-old Frost tetraploid.

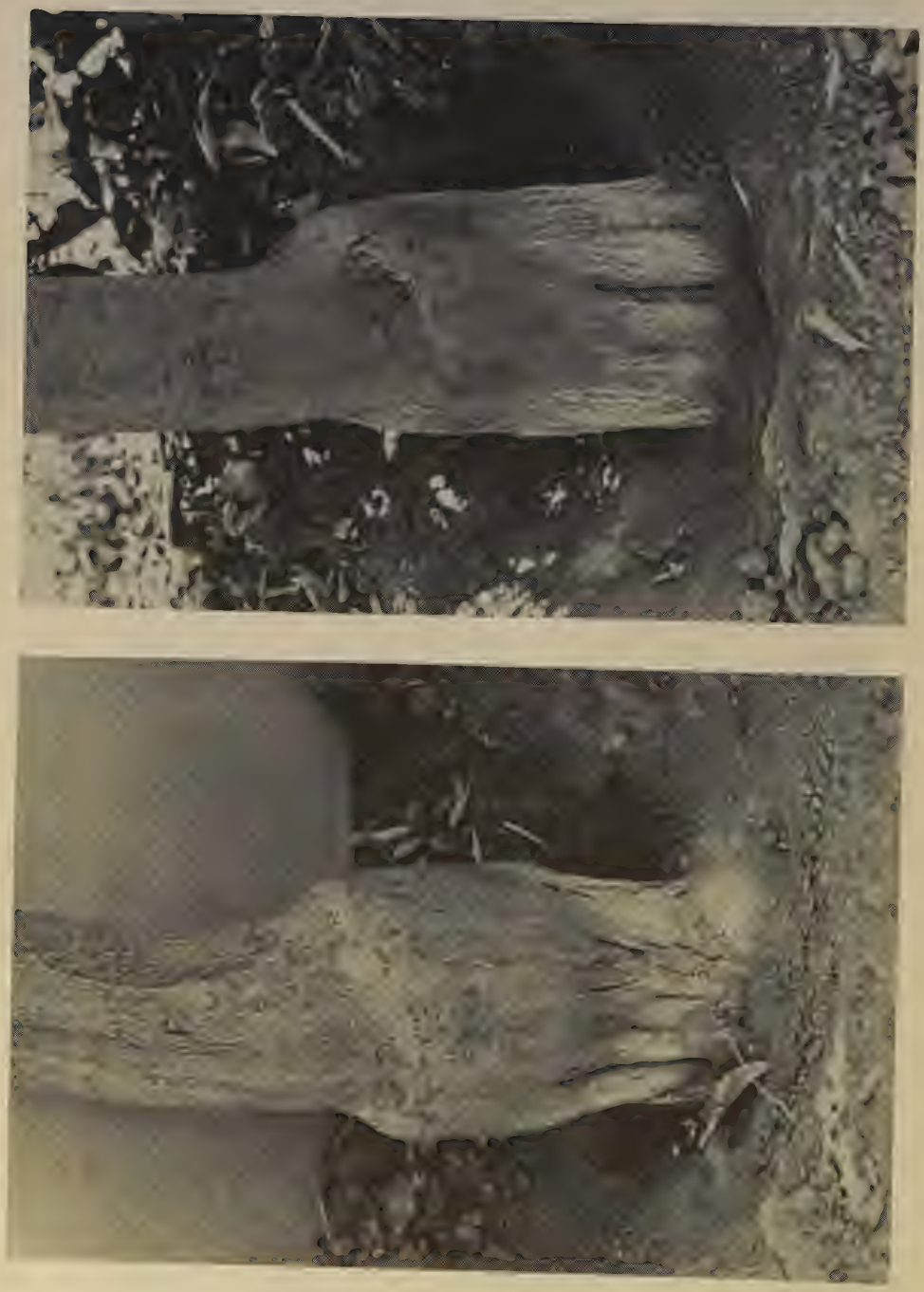

FIGURE 2. Scions of uninoculated 12-year-old seedling-line Wilking mandarin on trifoliate orange. Left -- On Frost diploid with subterranean bark cracks. Right -- On Frost tetraploid with normal bark.

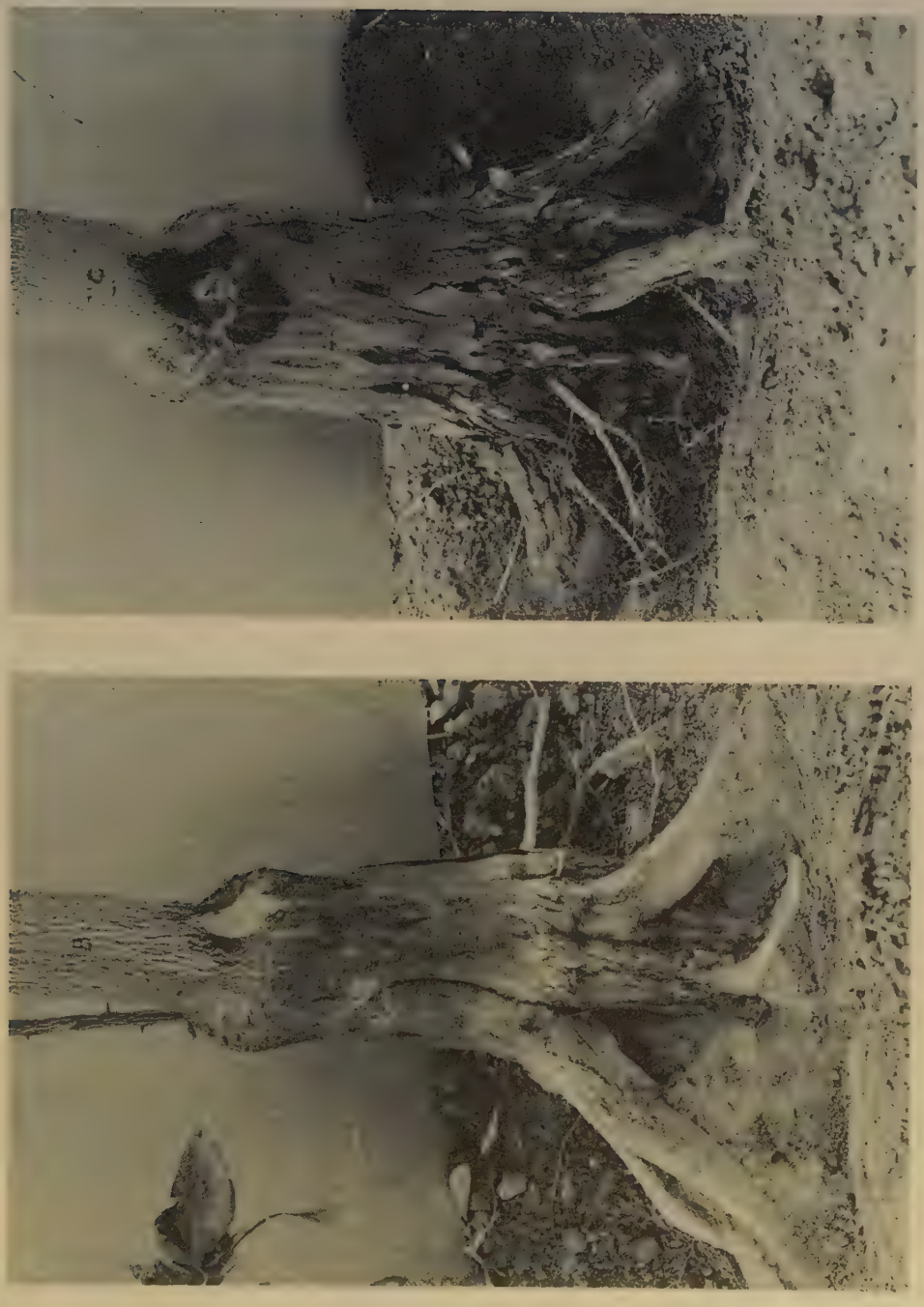

FIGURE 3. Exocortis-infected trunks of 12-year-old Temple tangor on trifoliate orange. Left -- Frost diploid rootstock. Right -- Frost tetraploid rootstock.

Table 1. Summary of exocortis symptoms and subterranean bark cracking in diploid and tetraploid Poncirus trifoliata rootstocks with indicated scions.

Scion Variety	Old-line (O) Young-line (Y) Scions	Number of diploid rootstocks			Number of tetraploid rootstocks		
		Examined	With bark cracks	With typical exocortis	Examined	With bark cracks	With typical exocortis
Allspice tangelo	Y	4	2	0	3	0	0
Clementine mandarin	O	4	4	0	3	0	0
Dancy mandarin	Y	3	3	0	3	0	0
Dweet tangor	Y	4	3	0	4	0	0
Honey mandarin	Y	4	4	0	3	0	0
Kara mandarin	Y	3	1	0	3	0	0
Kinnow mandarin	Y	3	2	1	4	0	1
Mency tangor	Y	4	3	0	3	0	0
Pearl tangelo	Y	4	4	0	4	0	0
Ruvel orange	Y	4	4	0	4	0	1
Satsuma mandarin 2-12	Y	3	3	0	4	0	0
Satsuma mandarin 3-8	O	2	0	0	2	0	0
Satsuma mandarin 3-9	Y	2	2	0	3	0	0
Temple tangor	O	4	0	4	4	0	4
Washington navel orange 1-11	Y	2	1	0	1	0	0
Washington navel orange 16-23	O	2	1	0	2	0	0
Wilking mandarin	Y	3	2	0	2	0	0
Wilp tangelo	Y	2	2	0	2	0	0
Totals		57	41	5	54	0	6

Detailed nursery plot records kept by H. B. Frost were available for all these trees. They show that in the nursery each of the Kinnow and Ruvel trees was grown 1 foot and 2 feet, respectively, in the direction of water flow from a Temple tangor; a Kara mandarin was grown 1 foot up the row in the opposite direction from each Temple tangor tree. None of the rootstocks having Kara mandarin scions has developed symptoms typical of exocortis (Table 1). After propagation of the scions in 1946 the young trees were held in the nursery for approximately 2 1/2 years before being planted in their present locations in the orchard. There, none of the Ruvel or Kinnow trees with typical exocortis is located next to a Temple tangor.

Except for the six trees with typical exocortis symptoms and one off-type plant, the trunks of the tetraploid trifoliate orange seedlings and rootstocks (Figs. 1 and 2) have remained consistently normal.

Bark samples were removed from the rootstocks of several trees, sectioned, and treated with a saturated solution of phloroglucinol in 18 percent HCl, as described by Childs, et al. (3). Reactions of free-hand sections of bark from aboveground and belowground areas of the diploid trifoliate trunks were exocortis-negative for the two diploid seedling trees tested and for the diploid rootstocks under tops of Clementine and Dancy mandarins, Pearl tangelo, and Mency tangor. Each of these trees showed bark cracking, but not typical exocortis. Mild exocortis-positive reactions were obtained in sections of diploid rootstocks under Temple tangor and the diseased Kinnow mandarin and in sections of tetraploid rootstocks under Temple tangor and the diseased Ruvel orange.

DISCUSSION

Fawcett and Klotz (4) suggested in 1948 that exocortis might be genetic in nature, or due to virus infection. Then, in 1950, Benton et al. (1) presented evidence that exocortis is a virus disease. Bitters et al. (2) in 1954, and numerous workers since that time, have confirmed that exocortis is a transmissible disease. The present report very probably deals with two disorders, the first of which, although it actually may be due to genetic factors, resembles exocortis, and the second of which obviously is transmissible exocortis.

In the first disorder abnormal cracking and necrosis of bark tissues in diploid P. trifoliata seedlings and rootstocks is noteworthy because a considerable number of plants is involved with symptoms resembling those of exocortis although circumstances and atypical symptoms indicate that the disorder probably is not exocortis. Possible explanations for the appearance of this unusual cracking in so many plants of a particular selection of trifoliate orange include: 1) Seed or graft transmission of exocortis virus; 2) Seed or vector transmission of a virus other than exocortis; 3) Genetic factors favoring bark cracking, possibly influenced by chemicals or by invasion of microorganisms, in a soil environment.

While in the absence of completed transmission tests the chance of seed transmission of exocortis cannot be overruled entirely, it is largely discounted by the facts that: 1) Considerable exfoliation has appeared on the lower trunks and primary roots of the diploid rootstocks growing under Temple scions known to carry exocortis virus, while very little scaling has occurred under other scions or in the diploid seedlings; and 2) The phloroglucinol-HCl test for exocortis was negative except in rootstock bark from the trees where the Temple scions are known to have carried exocortis virus, and from three other trees. Graft transmission of the exocortis virus to the diploid trifoliate, with a few obvious exceptions, is discounted by the absence of cracking and exfoliation in the exocortis-sensitive tetraploid rootstocks under 15 of the 18 scion groups and by the presence of bark cracks in the three diploid seedlings but not in the three tetraploid seedlings. Presence of a seed-transmitted virus other than exocortis could, of course, account for all the bark cracking which developed in the diploid trifoliate progenies, inasmuch as all affected rootstocks were seedlings of a similarly affected parent tree. Since no other selection of trifoliate orange rootstock with this type of cracking has been found nearby, vector transmission appears improbable unless these symptoms are expressed only on certain unusual genotypes of trifoliate orange.

Genetic factors appear to the authors to be the most probable reason for the development of bark cracking in this selection of diploid trifoliate orange, whether indirectly by increasing the sensitivity to a virus or to the soil environment, or directly by causing inherent anatomical abnormalities. A possible genetic disorder of this type may have little or no importance commercially, but its occurrence in plants being used for exocortis indexing would make the interpretation of symptoms on indicator rootstocks more difficult, whether the cause be genetic or a seed-transmitted virus.

The development of typical exocortis in three rootstocks having Kinnow mandarin or Ruvel orange tops appears to be due to infection contracted in the nursery, through root grafts or vectors, from Temple tangor trees propagated from buds of an old-line tree known to carry exocortis virus. Although it has not been determined whether the virus was transmitted by root grafts or otherwise, some of the trees grown near to and downhill from the exocortis carriers in the nursery became infected, while none of the trees with Kara mandarin tops, each grown only 1 foot uphill from the Temple trees, has developed exocortis (Table 1). This apparently unidirectional spread of the virus within the nursery suggests that exocortis transmission there may not have depended upon root grafts alone, but might have been accomplished by a vector which moved principally in the direction of water flow.

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INFLUENCE OF RING SPOT VIRUS ON GROWTH AND YIELD OF SOUR CHERRY

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Summary

Growth of Montmorency cherry propagated from buds infected by latent ring spot virus was up to 10 percent less than that of healthy trees. The difference in yield between the two kinds of trees was greater, the infected trees yielding approximately 20 percent less than the healthy ones.

Inoculation of healthy English Morello trees with recurrent ring spot virus caused an appreciable retardation of growth for 3 years, the duration of the experiment. Yield was reduced at least as much as was growth. Growth and yield of trees propagated from buds infected by latent ring spot virus were not affected by inoculation of the trees with recurrent ring spot virus.

Ring spot virus is very common in sour cherry orchards in New York State. In fact, it was necessary to index trees in very young orchards or in nursery plantings to find trees free of this virus for propagation purposes. Subsequent studies indicate that the virus spreads comparatively rapidly in the orchard but that characteristic ring spot leaf symptoms are uncommon on the trees except for a period of 1 or 2 years after individual trees have become infected. Roguing an orchard for control of the ring spot disease seems impractical (2).

Because of the reasons mentioned, information concerning conditions that influence the rate of spread of ring spot virus, the effect of the virus on growth and yield of the trees, and the relation of ring spot virus to other viruses that affect cherry is important to any control measures that might be devised. It is commonly known that one strain of ring spot virus already present in a tree will prevent shock symptom expression if the tree is inoculated with a different strain, but no data have been published on whether yield is affected by the challenge inoculation. Investigations on all these points are underway, and the present paper constitutes a progress report on part of that study.

LITERATURE REVIEW

H. J. Miller, in work reported by Lewis (2), obtained data indicating 11 to 37 percent less yield by 10- to 18-year-old Montmorency trees showing ring spot symptoms than by comparable trees that did not show symptoms. In later work in Pennsylvania, Lewis (3) found that yield was much below normal in the year that a tree showed shock ring spot symptoms but that in subsequent years the bearing capacity of affected trees approached that of healthy trees. Klos (1) obtained results in agreement with those of Lewis.

Milbrath (4), in propagation experiments, obtained poorer growth when he used Montmorency buds from trees affected with ring spot than when buds from healthy sources were used. Millikan (5) propagated the same clone of Montmorency in the virus-free state and after inoculation with ring spot virus. Infected budlings made substantially less growth than did the virus-free budlings but some strains of the virus were so mild that reductions in growth were detectable only during the middle part of the growing season.

Cross protection between strains of ring spot virus has been used in indexing of budwood sources for the determination of freedom from viruses (6). Evidence has been presented (7) that one strain of ring spot virus may displace another strain of the same virus in cherry trees.

EXPERIMENTAL

Plantings of Montmorency trees affected by ring spot and of healthy trees of the same variety were made to study the influence of ring spot virus on growth and yield of the trees, and spread of the virus in the orchard. Studies were also made of any possible relation of ring spot

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[1] Others who helped with certain phases of the work include E. J. Klos and R. W. Goodno. The advice and suggestions of A. Frank Ross have been very helpful.

virus to other viruses as they spread in the planting. These studies are cooperative among the two New York agricultural experiment stations and experiment stations in Pennsylvania and West Virginia, with comparable plantings made in the three States. The data presented in this report are limited to those obtained in the New York plantings on the influences of the virus on growth and yield.

Another type of experiment involves the comparison of growth and yield of trees propagated from healthy and from diseased sources; half of each lot were inoculated with a strain of ring spot virus different from that present in the propagative material. Data from one such experiment with the English Morello variety are presented.

Growth and Yield in Orchard Plantings

Plantings were made of healthy and diseased Montmorency trees in a replicated design (Figure 1). Additional healthy trees were planted to fill out each block for further studies on spread.

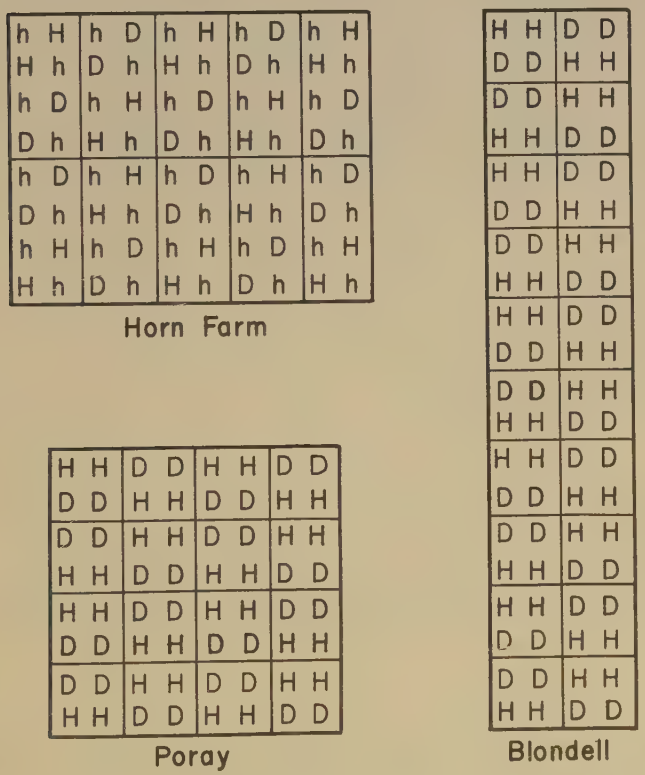

FIGURE 1. Charts showing arrangement of healthy (H) and ring spot affected (D) trees in orchard plantings. Two H and two D trees in each square or rectangle represent a replication. Trees in Horn Farm planting indicated by (h) are not included in the growth comparisons but were virus-free at planting.

Plantings

1) Horn Farm: The trees were planted in the spring of 1953. Originally, 40 virus-free (H) trees and 40 trees infected by ring spot virus (D) were planted in 20 two-tree replications. All were propagated on commercial Mahaleb rootstock, the H trees at Geneva and the D trees at Sodus. In 1954, two of the H trees indexed positive for virus on peach seedlings. Five H and three D trees were lost because of wet soil. One of the D trees showed a trace of ring spot in 1954 and one in 1958. Of the two H trees that indexed positive in 1954, one showed a traceof enations in 1956 and one showed shock ring spot in 1957. Data for only 10 replications (as shown in Figure 1) on the Horn Farm are included in Table 1 because of wet soil in part of the planting; the two H trees that indexed positive are in replications not included in the data presented.

2) Poray: The trees were planted in the fall of 1953. There were 32 each of H and D trees, all propagated at Geneva on virus-free Mahaleb rootstock obtained from the Canada Department

Table 1. Trunk growth[a] of healthy trees (H) and of trees infected by ring spot virus (D) in different plantings of Montmorency cherries.

Planting	Year	Trunk circumference: at beginning and end of test (cm) H	D	Increase in trunk circumference (cm) H	D	Ratio (D/H X 100)
Horn Farm	1953	3.33	3.40	1.54	1.40	90.9
	1954			4.21	3.76	89.3
	1955			6.21	5.67	91.3
	1956			4.14	4.22	101.9
	1957			5.72	5.69	99.5
	1958	29.81	29.49	4.67	5.36	114.8
Poray	1954	4.31	4.00	1.62	1.83	112.9
	1955			5.03	5.18	102.9
	1956			4.65	4.38	94.2
	1957			5.15	4.76	92.4
	1958	27.15	26.31	6.40	6.16	96.3
Blondell	1955	3.46	4.03			
	1955 and 1956			3.82	3.63	95.0
	1957			3.80	3.57	93.9
	1958	15.88	15.90	4.80	4.68	97.5

[a] Figures are averages of 20, 32, and 40 trees for the Horn Farm, Poray, and Blondell plantings respectively.

of Agriculture Laboratory of Plant Pathology at St. Catherines, Ontario. All H trees indexed negative on peach in 1954. One D tree showed a type of chlorotic mottle in 1956, and one H tree showed a trace of ring spot in 1958.

3) Blondell: The trees were planted in the spring of 1955. There were 40 each of H and D trees, all propagated at Geneva on virus-free Mahaleb obtained from Dr. Earle C. Blodgett, Irrigation Experiment Station, Prosser, Washington. All H trees indexed negative on peach in 1955, and no trees have yet expressed symptoms.

Results

Growth determinations were made on these trees by measuring the trunk circumference of each tree when planted and annually thereafter at the end of the growing season. The figures given are averages for the group determined from replication averages.

At two of the plantings, growth of healthy trees in the early years was greater than growth of diseased trees; at one, the reverse was true. It is interesting that the healthy trees at the Horn Farm planting grew more than the diseased ones in the early years, that in 1956 and 1957 growth was approximately equal in the two lots, and that in 1958 the diseased trees made more growth than the healthy ones. The first crop of fruit was in 1958 when the average yield on the healthy trees was 57.3 pounds per tree and on the diseased trees 45.4 pounds, a difference of 21 percent. The estimated apparent set of fruit (fruit that reached maturity) independent of size of tree was greater on healthy than on diseased trees. Fruit size was equal on both kinds of trees.

Trees at the Poray planting produced an estimated 5 to 10 pounds per tree in 1958, with a slight difference in favor of the virus-free trees in the estimates made.

Cross Protection and Influence on Growth and Yield

In 1954, English Morello trees were grown at Geneva on virus-free Mahaleb rootstocks and planted in the fall in a replicated design at Sodus. Rows were 6 feet apart and trees were 4 feet apart in the row. Half of the trees were propagated from buds containing latent ring spot and the other half with virus-free buds; the two bud sources were obtained from different nurseries. Trees within each kind were paired, and one of each pair inoculated in August 1955 with ring spot virus.

All trees that were healthy when inoculated developed recurrent ring spot symptoms. Some of the diseased trees expressed variable symptoms the first year (1956), but no ring spot symptoms could be found on them in 1958 regardless of whether they had been inoculated.

Circumference measurements have been made on the trunks of these trees each year, beginning in the fall of 1955 soon after the inoculations were made. Yield and fruit size measurements were made only in 1958, although fruit has been borne each year. The data shown in Table 2 are averages of 14 single-tree replications.

Table 2. Growth and yield of a 1954 planting of English Morello trees propagated from healthy and from diseased (latent ring spot) buds, with and without inoculation with recurrent ring spot virus in 1955.

Status of tree[a] or comparison made	Trunk circumference (cm)				Yield (ounces)		Fruit size
	At start (August, 1955)	Increase 1956	Increase 1957	Increase 1958[b]	Per tree	Per cm trunk circumference	Ounces/100 fruits
H	6.06	5.17	3.05	1.03	114.75	7.50	14.59
Hi	6.03	4.84	2.51	0.81	96.25	6.79	15.23
D	5.12	4.20	2.62	1.15	67.25	5.12	14.19
Di	5.06	4.00	2.56	1.09	65.55	5.13	13.61
	(Ratio X 100)						
Hi/H	99.5	93.6	82.3	78.6	83.9	90.5	104.4
Di/D	98.8	95.2	97.7	94.8	97.5	100.1	95.9
D/H	84.5	81.2	85.9	111.7	58.6	68.3	97.3
Di/Hi	83.9	82.6	102.0	134.6	68.1	75.6	89.4

[a] H -- healthy; Hi -- originally healthy, inoculated in 1955; D -- propagated from a source containing latent ring spot; Di -- same lot of trees as those in (D), inoculated as were the Hi trees.

[b] Measurement made in August; hence, growth increment for the year small.

The recurrent ring spot virus caused a reduced yield and retarded growth of the originally healthy trees (Table 2). This effect on growth was progressive, becoming greater each year. In contrast, inoculation of the diseased trees with recurrent ring spot virus affected neither growth nor yield; however, the originally healthy trees outyielded trees propagated from diseased buds, even when those of the former group were inoculated. This is true of both the actual yield per tree and the yield per cm of trunk circumference.

DISCUSSION

It appears that although some strains of ring spot have comparatively little influence on growth of the trees, they may have a marked effect on fruit set. In both the Montmorency trees planted under orchard conditions and the English Morello in closely planted experimental rows there is evidence that when the trees come into bearing this differential in growth may be overcome. Diseased trees make growth equal to or greater than that of healthy trees, probably because of the lighter crop. If comparisons are made on the basis of tree size, with fruiting not being taken into account, this phenonemon might lead to incorrect conclusions.

Apparently one virus strain may protect against another, but these data indicate some of the milder strains of ring spot virus may be more damaging than is at first evident. The data on fruit set are in agreement with those of Klos (1) in work on mature orchard trees. If use is to be made of cross protection as a control measure, milder strains than those used here must be found.

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EFFECT OF TWO VIRUS COMPLEXES ON THE RESPONSES OF TWO STRAWBERRY VARIETIES

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Abstract

Plants of Catskill and Blakemore varieties of strawberry free of all except latent A virus were graft-inoculated with either mottle virus or with latent C plus leaf curl virus. In replicated field plots, plants infected with these complexes produced fewer runner plants, lower yields, and smaller fruit than those infected with latent A only. The virus complexes affected the two varieties differently; mottle complex was more severe in Blakemore and the other in Catskill.

INTRODUCTION

Several investigators have compared virus-free and common stocks of strawberries (3, 4, 8, 10, 11, 12). Observed differences have generally been in favor of the virus-free stock. Fulton (9) noted that virus adversely affected both vigor and runner plant production of the Blakemore variety.

Becker and Rich (1) in a detailed report showed that of the three varieties they tested, virus-free Catskill and Premier produced more runner plants. Yields of virus-free clones of all three varieties were significantly larger; however, the authors attributed the greater fruit yield of the virus-free clones to a larger number of runner plants than to more or larger berries per plant. Moulton et al. (14) attributed the higher yield of virus-free Catskill to earlier runner plant formation.

Comparisons of virus-infected clones and clones heat-treated to eliminate viruses are reported from England and Switzerland. In both reports, plants were grown in hill culture and plant vigor as well as yield were recorded. Rogers and Fromow (16) noted that several varieties freed from certain viruses by heat therapy grew more vigorously. Yields of several varieties were higher, but those of two were lower. Fruit of treated clones was later ripening and had greater tendency to develop Botrytis gray mold rot. Bovey (2) noted significant differences in yield but none in average fruit size of the Madame Moutot variety free-of virus and infected with two kinds of virus.

The present study reports the responses of two varieties experimentally graft-inoculated with virus complexes.

MATERIALS AND METHODS

During 1955, plants of the strawberry varieties Blakemore and Catskill carrying only the latent A strain of virus (7) from the East Malling clone of Fragaria vesca (EMC) were graft-inoculated with known viruses. The viruses selected for inoculation were those which reproduce the two symptom types described by Demaree and Marcus (5) as being commonest in strawberries from the eastern United States. The simplest form of Demaree type 1 is a complex consisting of latent A plus mild mottle (13). Hereafter in this paper the mottle complex is referred to as V-1. The source of mild mottle used was supplied by N. W. Frazier. Source of the type 2 virus complex was a clone of the variety Big Joe previously found to carry latent C (13) plus a virus similar in symptom expression in F. vesca to leaf curl virus (Prentice virus 5) (15). Hereafter in this paper this particular complex is referred to as V-2.

Inoculation was by runner graft to the first runner produced by a mother plant. Subsequent runner plants were potted as they developed. Transmission of virus was confirmed by grafting daughter plants to EMC several months after removal of the graft from the virus-source plant.

Plants of the two latent A-infected clones and the four inoculated clones were not comparable in age or condition when planted in the field in the spring of 1956. Some were pot-bound and some very succulent; and the resultant matted beds showed differences obviously not attrib-

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utable to virus content.

Therefore, in the spring of 1957 runner plants from these plots were used to set new plots. A split-plot design with four plants per 15-foot sub-plot in five replications was used. Plants were allowed to form matted rows without removal of runners. Aisles were maintained by normal cultivation. Throughout the 2 years these plants were in the field, they were dusted frequently with parathion to prevent aphid spread of virus.

RESULTS

Runner plants were counted on three dates during the summer of 1957. These counts were analyzed, the means were compared by Duncan's multiple range test (6), and appear in Table 1. Catskill V-1 produced more early runners than Catskill A, but by the end of the season there were no statistical differences between the numbers of runner plants. Runnering of Catskill V-2 was severely depressed throughout the season. Blakemore A produced a highly significant greater number of runners than either Blakemore V-1 or Blakemore V-2 on all dates.

Table 1. Runner plant counts on three dates of Catskill and Blakemore strawberry plants infected with different viruses, 1957, Beltsville, Maryland.

Variety and virus type[a]	July 10[b]	July 24	August 9
Catskill V-2	8.4	18.4	25.4
Catskill A	18.0	34.0	46.6
Catskill V-1	26.4	37.2	45.4
Blakemore V-1	26.8	38.6	59.2
Blakemore V-2	30.6	46.6	69.4
Blakemore A	70.4	81.6	107.8

[a]A = Latent A virus; V-1 = latent A plus mild mottle virus (type 1 virus complex); V-2 = latent A plus latent C plus leaf curl virus (type 2 virus complex).
[b]Multiple range test. Brackets enclose values which do not differ significantly at 5 percent level.

Table 2. Mean yield in pounds per plot of fruit of Catskill and Blakemore strawberry plants infected with different viruses, 1958, Beltsville, Maryland.

Variety and virus type[a]	Yield	5 percent[b]
Catskill V-2	3.0	
Blakemore V-1	5.5	
Blakemore V-2	6.9	
Catskill V-1	9.0	
Blakemore A	9.6	
Catskill A	10.7	

[a]See Table 1.
[b]See Table 1.

In the spring of 1958, fruit was harvested in six pickings between May 29 and June 13 and the yield from each plot was weighed. These data appear in Table 2.

There were obvious differences among treatments at time of harvest. To determine whether the viruses had an effect on yield other than merely depressing vigor, two methods were used to measure the vigor for comparison of yields: the runner plant counts on August 9 and an estimate of area in square feet of plants in each plot.

In the first comparison, the formation of more late runner plants by the Blakemore A and Blakemore V-1 with resultant competition may explain the lower yield of these two; however, differences among the three Blakemore treatments were not significant. Differences among

the Catskill treatments that appear in Table 3 demonstrate that the decrease in yield resulting from the type 2 complex was greater than could be attributed to runner inhibition.

In the second comparison, the area in square feet covered by plants in each plot was estimated. This was used to calculate the yield per square foot of plant bed produced (Table 4). There was no statistical difference among the three Blakemore treatments, but again the decrease in yield of Catskill V-2 was greater than could be attributed to runner inhibition.

Table 3. Mean yield in pounds of fruit per runner plant formed by August 9, 1957 of Catskill and Blakemore strawberry plants infected with different viruses, 1958, Beltsville, Maryland.

Variety and virus type[a]		Yield	5 percent[b]
Blakemore	A	.092	]
Blakemore	V-1	.105	]
Blakemore	V-2	.118	]
Catskill	V-2	.132	]]
Catskill	V-1	.196	]
Catskill	A	.234	]

[a]See Table 1.
[b]See Table 1.

Table 4. Mean yield in pounds of fruit per estimated square foot of plant bed of Catskill and Blakemore strawberry plants infected with different viruses, 1958, Beltsville, Maryland.

Variety and virus type[a]		Yield	5 percent[b]
Blakemore	V-1	.216	]
Blakemore	A	.218	]
Blakemore	V-2	.232	]
Catskill	V-2	.280	]
Catskill	V-1	.390	]
Catskill	A	.452	]

[a]See Table 1.
[b]See Table 1.

Table 5. Mean number of berries per pound for the first three pickings of Catskill and Blakemore strawberry plants infected with different viruses, 1958, Beltsville, Maryland.

Variety and virus type[a]		Berries per pound (number)	1 percent	5 percent[b]	Size reduction[c] (percent)
Catskill	A	44.4	]	]	0
Catskill	V-1	56.6	]	]	22
Catskill	V-2	85.0	]	]	48
Blakemore	A	85.8	]	]	0
Blakemore	V-2	111.6	]	]	23
Blakemore	V-1	142.8	]	]	40

[a]See Table 1.
[b]Multiple range test. Brackets enclose values which do not differ significantly at the indicated levels.
[c]As compared with the latent A treatment of the same variety.

When the yield per plot at each picking was 1/2 pound or more, the number of berries per pound was recorded. There was insufficient yield from many plots from the fourth picking on; so data on the average size of fruit for only the first three pickings were used in Table 5. The reduction in fruit size also appears in Table 5.

DISCUSSION AND SUMMARY

Comparison of the responses within varieties shows the effects of the two virus complexes.

Catskill: A vs. V-1. The only difference was a reduction in berry size by the type 1 virus complex at the 5 percent level.

A vs. V-2 and V-1 vs. V-2. In Catskill the V-2 virus complex resulted in lower (5 percent level) runner-plant production, lower (1 percent level) total yield, lower (5 percent level) yield per square foot of bed, and smaller (1 percent level) berries as compared with A or V-1. The V-2 virus complex resulted in a lower (5 percent level) yield per plant formed by August 9 compared with A, but no difference compared with V-1.

Blakemore: A vs. V-1. V-1 virus complex resulted in lower (1 percent level) runner-plant production, lower (1 percent level) total yield, and smaller (1 percent level) fruit size, but no difference in yield per square foot of bed or yield per runner plant formed by August 9.

A vs. V-2. V-2 virus complex resulted in lower (1 percent level) runner-plant production, lower (5 percent level) total yield, smaller (1 percent level) fruit size and, again, no difference in yield per square foot of bed or per runner plant formed by August 9.

V-1 vs. V-2. The only difference was a reduction of berry size at the 1 percent level by the V-1 virus complex.

The advantage of the generally superior vigor and greater runner production of virus-free stocks over common stocks, which has been recognized by the strawberry nurserymen, is demonstrated again by the data presented.

That the advantage of virus-free stock may extend to the fruit grower is demonstrated by greater yields, resulting from an increased number of runner plants, and the larger fruit size in spite of more competition among plants.

Varieties may differ markedly in their response to a given virus complex. Of the particular virus complexes used in this experiment, V-1 was more severe in Blakemore and V-2 in Catskill. The magnitude of differences in yield in Catskill infected with V-2 virus complex is greater than the difference in number of plants.

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TOBACCO NECROSIS VIRUS IN CLEOME

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Abstract

Infection of the roots with a strain of tobacco necrosis virus (TNV) has been associated with increased plant height or weight of spider plant (Cleome spinosa L.), bean, cotton, and cucumber. No increase in growth of radish, tomato, cowpea, calendula, sweet william, primrose or poppy as a result of root infection with this virus has been observed. The drainage water from pots containing TNV-infected Cleome contained an abundance of the virus. When the leaves of healthy Cleome plants were inoculated with TNV, typical lesions resulted, but Cleome plants bearing virus in the roots also contained the virus in the leaves but showed no leaf symptoms.

METHODS

The strain of TNV principally used, hereafter called Cleome TNV, was from soil secured through Dr. A. H. Gold from Salinas, where TNV was first found in California (15). The field from which the soil came was in potatoes in 1958 and was originally of interest because of potato rattle virus in this field (7). However, the virus under study is not tobacco rattle virus, and there is no good reason to believe that rattle virus played any part in the results presented. The virus under study is considered to be TNV because the pathology and symptoms of infection with this virus on bean, cowpea, cucumber and tobacco are similar to those shown by designated strains of this virus from Madison, Wisconsin (6) and at Cambridge, England (11, 12).

Cleome spinosa L. (spider plant, variety Pink Queen) was used as a host because Dr. L. M. Black had indicated to me that Cleome was commonly naturally infected with TNV near Urbana, Illinois. Cleome was started from seed and transplanted as seedlings to 4-inch pots of sterilized compost soil. The other host plants were mostly seeded directly in 3-inch or 4-inch pots. To prevent natural infection of the roots with TNV, which was known to be present on some of the greenhouse benches (4), the regular 4-inch pots of plants were placed within glazed 4-inch pots, the bases of which were waterproof. These glazed pots also permitted easy collection of drainage water from the pots.

In the five tests with Cleome referred to here, inoculation was by three different methods. In the first, about 10 percent of the Salinas soil was added to sterilized soil in which the plants were to be grown. While no sterilized Salinas soil was added to the control pots, it is not considered that the Salinas soil as such was responsible for the growth effects noted since similar responses resulted when the virus was added as leaf or root extract. In another test on Cleome, inoculation was by pouring about 25 ml of the supernatant of a suspension of the Salinas soil on the soil in which Cleome was growing. In this case again controls consisted of pots to which no soil suspension was added. In most tests with Cleome and other plants, inoculum consisted of a water suspension of macerated cowpea leaves or Cleome roots which were infected with TNV. This suspension was poured on the soil surface or in depressions in the soil in which test plants were growing. Controls consisted of pots to which a comparable suspension of healthy leaves or roots was added.

Although the plants were originally started in sterilized soil, no precautions were taken to maintain sterility, and it is assumed that the soil was quickly and thoroughly contaminated with microorganisms from the environment and from the inoculum.

Since the roots showed no recognizable symptoms, infection in roots was determined by local lesion assay on primary leaves of bean or cowpea or on cotyledons of cucumber by standard methods (16). Sometimes the roots projecting through the drainage hole of the inner pot were assayed, sometimes the plants were sacrificed and the roots within the inner pot were assayed, and sometimes the drainage water was assayed. Such assays usually yielded 0 to 100 lesions on a single bean or cowpea leaf, but it is believed that the assay method is relatively crude and that infected plants are sometimes missed by this assay.

RESULTS

Symptoms

No symptoms of infection, other than plant size, were observed on plants of which only the roots were inoculated with Cleome TNV. However, the leaves of Cleome, as of many other plants (8, 11, 12) were highly susceptible to direct inoculation with this virus. Lesions on Cleome leaves were similar to those formed on cowpea, except that chlorosis extending far beyond the lesions was conspicuous on Cleome but not on cowpea. Only three lesions resulted from the inoculation of five Cleome stems.

The following results with Cleome TNV illustrate the well known fact that TNV is injurious to plant growth if the leaves are inoculated. Some cowpeas seeded December 8 were inoculated on the leaves December 20, and some were maintained as controls. At intervals control and inoculated plants were detached at the soil level and weighed. The green weight of the inoculated plants as a percentage of the green weight of the healthy plants was 86 at 3 days after inoculation, 42 at 9 days, 28 at 11 days, and 14 at 14 days after inoculation. It is believed that similar results might be secured with Cleome.

In most cases the lesions on cowpea resulting from the assay of Cleome roots were of the same type, and this type was distinct from two other single lesion isolates of TNV which were isolated direct from the Salinas soil. It would appear that only one of three types of TNV in the soil multiplied in Cleome roots and/or that the other two types had been modified by host passage (11, 13, 14). This type which was isolated from soil by means of Cleome is called Cleome TNV and was used in most tests reported here.

Growth

In the first trial with Cleome, the roots of plants about 10 cm tall were inoculated on November 7 and assayed on December 5. On December 9 the average height of the four plants without virus in the roots was 25, 28, 28, and 30 cm (average 28 cm) and the height of two plants which had TNV in their roots was 35 and 38 cm (average 36 cm). In the second trial the average height of seven control plants was 11.6 cm and the average height of five infected plants was 13.7 cm. In the third trial the average height of nine control plants was 14.7 cm and the average height of five infected plants was 16.6 cm. The plants were not weighed but the form of the control and infected plants was similar, and it is believed that the green weight was approximately proportional to the height.

In the first greenhouse trial on bean since the height-stimulating effect was noted on Cleome, Pinto beans, seeded December 4, were inoculated in the soil on December 20 while the plants were still in the primary leaf stage. On January 10 the height of five control plants was 28, 31, 28, 22, and 25 cm while the height of five plants inoculated with Cleome TNV was 41, 40, 43, 56, and 48 cm. The height of five plants inoculated with another strain of TNV was not clearly different from the controls.

In two out of two subsequent trials with Cleome, three out of four with cucumber, two out of three with bean, two out of two with cotton, and two out of two with barley, the average height or weight of the TNV-inoculated plants was greater than that of control plants. TNV was not recovered from barley roots.

Virus Assay

Dr. A. H. Gold had previously detected TNV by direct assay of supernatants of this Salinas soil. In the present study virus was detected in plant roots of inoculated plants, in soil of pots bearing infected plants, in the drainage water of pots bearing infected plants, and in leaves of some plants with infected roots. Only inoculated plants assayed positive for virus, but not all inoculated plants were positive.

Only pots bearing plants with infected roots yielded filtrate with virus; and since some inoculated plants did not become infected, the recovery of virus in the filtrate was clearly from the infected roots and not direct from the inoculum that had been added to the soil. The apparent absence of virus in some inoculated plants could have been because of failure of infection, because of localization of the virus in infected plants, or because of low virus titer in some infected plants.

The virus content of infected roots, of the drainage water from pots with infected plants, and of leaves of plants with virus in the roots was always less than the virus content of com-

parable leaf-inoculated cowpea or bean leaves. In one representative trial the drainage water from two infected plants applied to cowpea leaves yielded 2.6 and 4.9 lesions per square centimeter (306 lesions on four leaves) while 1 percent tissue in water of cowpea leaves bearing 12 and 15 lesions per square centimeter yielded 49 and 55 lesions per square centimeter, respectively.

Virus has as yet been detected in the leaves of the only two Cleome plants which were assayed at 52 days after the roots were inoculated. This is believed to be unusual for TNV since Smith (11) found that the symptom-free leaves of plants with TNV in the roots were consistently free of the virus. Leaves of five Cleome plants assayed at 30 days after the roots were inoculated yielded no virus.

DISCUSSION

Increased plant growth due to mycorrhiza fungi (10) and root nodule bacteria (5) is well known, but no case of over-all increased growth due to a virus infection has come to my notice. The protective action of a mild strain of a virus against a more severe strain of the same virus (3) might be considered as increased growth due to a virus infection but is certainly a special case. The increased growth of Cleome, bean, cucumber, and cotton, apparently as a result of a single infection with a strain of tobacco necrosis virus, is therefore of interest.

This preliminary and tentative report implies that crop yield might be increased as a result of virus infection. Publication of this report on the basis of such limited evidence is justified only by the importance of this implication. While TNV is recognized as a common innocuous parasite of the roots of many plants, it is known to be injurious under field conditions only to tulips and beans (2). In beans the virulence is known to increase with host passage (11, 13, 14) while its virulence to tobacco was decreased by passage through bean (13, 14). The recognized injury from TNV as observed on beans in England (12) and in Holland (2) has been duplicated in part in the greenhouse in the present study, in that in beans inoculated as seedlings with another strain of TNV, the virus moved very slowly up the stems of some of the plants, caused necrosis, and eventually killed some of the plants. This was in spite of an apparent early increase in growth without necrosis.

This further indicates that with TNV as with root nodule bacteria (5) and with mycorrhiza fungi (10) there are strains of the infectious entity differing in injurious effect, and there are environmental conditions under which infection is injurious, as well as conditions under which it increases plant growth. Details of the conditions under which increased growth occurs remain to be worked out. Scientifically the interesting finding is that the same parasite in the same plant may be injurious if introduced by mechanical inoculation into leaves, non-injurious if introduced into leaves as a result of inoculation of the roots, relatively non-infectious if introduced into stems, and stimulatory if introduced into roots.

The presence of TNV in Cleome leaves without symptoms is similar to the occurrence of TNV in Primula leaves without symptoms (1, 9), but with Primula direct inoculation of the leaves did not yield symptoms, whereas direct inoculation of Cleome leaves yielded typical necrotic lesions.

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CRACKING AND ROT CONTROL OF SWEET CHERRY[1]

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Abstract

Twelve fungicidal chemical compounds or combinations of chemicals applied to Windsor sweet cherry resulted in differences of 12 to 96 percent in amount of cracked fruit. Other differences noted were in fruit size, maturity and rot control. A combination of low soluble copper, wettable sulfur and lime gave the least cracking, while highly surfactant materials appeared to give the most. Copper-sulfur-lime, ferbam-sulfur-glyodin, Acti-dione-glyodin, and sulfur-glyodin plots showed the least brown rot. Acti-dione dwarfed the fruit and delayed maturity. The copper spray reduced fruit size and gave objectionable residue but was the most profitable application for the processing market.

In a routine test of materials and combinations of materials for control of brown rot (Monilinia fructicola) and cherry leaf spot (Coccomyces hiemalis) of sweet cherry, a wide range in amount of fruit cracking resulted. Differences for fruit size, maturity and rot (Monilinia, Botrytis and Rhizopus spp.) control were also noted. Leaf spot was not present.

Four plots received glyodin alone and in combination, namely, glyodin, 1 quart; glyodin 1 pint plus sulfur 5 pounds; glyodin 1/2 pint, plus ferbam 3/4 pound plus sulfur 2 1/2 pounds; and glyodin 1/2 pint plus Acti-dione 1 ppm. Three plots received Cyprex and its acid phthalate and borate salts at 3/4 pound each. The other plots compared captan and Phaltan at 2 pounds each plus ML 104 sticker; Thylate 1 1/2 pounds; and a copper-sulfur-lime mixture of 1 1/2 pounds Tenn. 26 copper plus 2 1/2 pounds sulfur plus 3 pounds spray lime. The check plot received insecticides only (DDT and parathion) which were also applied equally and separately to all test plots. The adjacent grower-sprayed trees received six sprays, the first three of ferbam and sulfur and the last three of captan.

The comparisons on Windsor sweet cherry were made from four applications at approximately 2-week intervals using a hand gun at 400 pounds pressure and sprayed thoroughly to the dripping point, beginning at petal fall and ending June 26.

Following the two rainy days of June 14 and 15 large differences in the amount of fruit cracking were readily seen in the differently sprayed plots. One quart samples were picked at random from each plot on July 16 and the counts were made July 17. The samples were held and photographed on July 22 when the checks appeared 100 percent infected with brown rot and the treated samples were showing rot control proportional to the original counts.

The data are presented in Table 1. The copper-sulfur-lime sprayed fruit showed the least cracking and resulted in the most profitable crop, with 87 percent of the fruit salable. The fruit was mature, and of a uniformly dark color desired by canners. A heavy, unsightly residue made the fruit objectionable for fresh fruit sales. The reduction of fruit cracking from the use of copper, calcium or aluminum salts and also weak bordeaux has been noted by others[4].

The Acti-dione-glyodin treatment, consisting of three sprays only (omitting the petal-fall spray), is contraindicated for early applications on sweet cherries. This spray reduced the size, delayed maturity, and distorted some of the fruits. It also resulted in leaf injury noticeable as bronzing of surface cells and cupping of the leaves.

The Phaltan-sprayed trees showed some scattered leaves with brown dead spots, perhaps of minor importance. The Phaltan-sprayed trees had the largest fruits, that were well matured and holding rots well 1 week after picking.

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Table 1. Effect of certain fungicides on fruits of Windsor sweet cherry.

Materials	Fruits (number)	Cracked fruits (percent)	All rots (percent)	Brown rot (percent)	Average weight single fruit (ounces)	Gain or loss (percent)	Maturity estimate
1. Copper-sulfur-lime	135	13.0	0.7	0.0	.186	-7.0	full-ripe
2. Phaltan-sticker	69	29.0	11	4.3	.236	16.0	ripe
3. Sulfur-glyodin	71	40.0	1.4	1.4	.215	7.0	med. ripe
4. Grower's program	70	41.5	1.4	1.4	.221	10.0	ripe
5. Thylate	78	67.0	5.1	0.0	.200	0.0	ripe
6. Cyprex (phthalate)	79	71.0	21	6.3	.200	0.0	ripe
7. Captan-sticker	71	72.0	17	4.2	.218	9.0	ripe
8. Ferbam-sulfur-glyodin	79	74.0	6.3	0.0	.202	1.0	med. ripe
9. Acti-dione-glyodin	80	77.0	6.2	0.0	.175	-12.5	green
10. Check (insect. only)	72	82.0	23	22.0	.201	0.0	med. green
11. Cyprex	74	88.0	22	5.5	.206	3.0	ripe
12. Glyodin	80	93.0	15	2.5	.203	1.5	ripe
13. Cyprex (Borate)	91	96.0	33	10	.181	-10.0	ripe

The sulfur-glyodin spray appeared to good advantage in preventing brown rot. The fruit was less mature than fruit on most of the other spray plots, but not less than the checks. It may well be that the early maturity as seen in some plots is an abnormality resulting from obscure injury.

It may be of significance that the surfactant materials, glyodin and Cyprex, resulted in the most cracking in this test. These results point to a need for further investigation of fungicides, insecticides and additives for use in sweet cherry sprays, particularly in their effect on cracking of the fruit following wet weather.

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TREATMENT OF FIELD BOXES FOR THE CONTROL OF POST-HARVEST ROTS OF PEACHES AND STORAGE ROTS OF APPLES[1]

Robert E. Adams and S. E. Tamburo[2]

Abstract

Treating field boxes with several fungicidal chemicals provided sufficiently effective control of storage rots of apple and post-harvest rots of peaches to be of practical importance.

INTRODUCTION

Storage rots of apples frequently cause the loss of 10 percent or more of the apples in refrigerated storage. Some of the infections take place while the fruits are on the tree, but this does not account for the relatively high incidence of storage rots encountered. The role of harvesting and handling procedures in the incidence of storage rots is not fully understood.

In investigations on the life histories of apple-rot pathogens in West Virginia, it was found that many of the fungi previously reported[3] occurred in or on field boxes commonly used for harvesting and storing apples. This suggested the possibility that field boxes serve as an additional source of inoculum. Since peaches generally are more susceptible to post-harvest decays than are apples, it seemed likely that field boxes also serve as an important source of inoculum for post-harvest rots of peaches. The purpose of this paper is to present evidence that field boxes are an important source of inoculum that may be partially eliminated by treating the boxes with fungicides.

MATERIALS AND METHODS

Apple Experiments

The apples used in these experiments were grown under the Captan-Lead spray program, recommended for use in West Virginia, at the West Virginia University Experiment Farm, Kearneysville, West Virginia.

The field boxes selected for these experiments had been used for two or three seasons prior to these experiments and had been well cared for. They had been stored inside the station barns when not in use.

In 1957 nine different treatments were applied to the boxes used for harvesting and storing Red Delicious and Golden Delicious apples, using a randomized block design, replicated five times. A total of 90 boxes was used, 45 for each variety. To assure uniform inoculum, the boxes in eight of the treatments were sprayed with a spore suspension of 12 fungi frequently isolated from storage boxes. This spore suspension contained a mixture of approximately 100 spores per milliliter each of Alternaria tenuis, Epicoccum granulatum, Nigrospora oryzae, Fusarium sp., Cladosporium cladosporioides, Phoma pomi, Botryosphaeria ribis, Physalospora obtusa, Chaetomium globosum, Trichothecium roseum, Botrytis cinerea, and Rhizopus nigricans. Five hundred milliliters of the suspension was sprayed into each box with a knapsack sprayer. After contamination the boxes were allowed to dry 4 to 5 hours and then were sprayed, with a knapsack sprayer, each to the point of run-off with one of the chemicals shown in Table 1.

During and after the contamination spraying, the naturally-contaminated check boxes were kept well away from the inoculum. The Red Delicious apples (tree run) were put into the boxes by the pickers, and immediately placed in refrigerated storage. The Golden Delicious fruits were hand sorted to remove all diseased fruits and then placed in the refrigerated storage. Counts of all the diseased and healthy fruits in each box were made on December 16-17,

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[3]Adams, R. E., and S. E. Tamburo. 1957. The West Virginia spot-rot complex of apple in 1956. Plant Disease Reptr. 41: 760-765.

Table 1. Treatments applied to boxes used for harvesting and storing Red and Golden Delicious apples in 1957.

Treatment	Material	Concentration
artificially contaminated	mercuric chloride	1:1000
artificially contaminated	cycloheximide	5 ppm
artificially contaminated	copper sulfate	4 lb. in 100 gal. water
artificially contaminated	captan	2 lb. in 100 gal. water
artificially contaminated	glyodin	1 qt. in 100 gal. water
artificially contaminated	maneb	2 lb. in 100 gal. water
artificially contaminated	zineb	2 lb. in 100 gal. water
artificially contaminated	sterile water check	
naturally contaminated	sterile water check	

1957, after approximately 2 1/2 months of storage.

In 1958 two series of experiments were made. The first consisted of the application of fungicidal sprays to field boxes, in a manner similar to that in 1957, except that the boxes were not artificially contaminated with spores of pathogenic fungi. The treatments used were: captan, 2 pounds in 100 gallons water; zineb, 2 pounds in 100 gallons water; amobam, 1 quart in 100 gallons water; glyodin, 1 quart in 100 gallons water. New, unsprayed boxes and used, unsprayed boxes were used for comparison. The varieties of apples used were Rome Beauty and Stayman Winesap. The experimental design consisted of six treatments in randomized blocks, replicated nine times for each variety. The second series consisted of the same four fungicidal treatments applied to the boxes as a dip rather than as a spray. This experiment was laid out in a randomized block design of five treatments, replicated five times. A total of 158 boxes was used in these two experiments. The fruits were harvested directly into the boxes by the pickers, and placed in refrigerated storage October 15. Counts of diseased and healthy fruits were made January 2, 1959, after approximately 2 1/2 months of storage.

Peach Experiments

Studies with Hale Haven peaches similar to those made with apples were conducted in two separate localities (Kearneysville and Romney) during 1958. These experiments were laid out in a randomized block design of six treatments, replicated five times in each locality. The spray treatments applied to the boxes were the same as those used for the apple boxes in 1958, except that paste sulfur (5 pounds actual in 100 gallons) was substituted for the glyodin treatment, and tray-pack, cardboard liners were used in place of new boxes. The fruit was put into the treated boxes by the pickers in the commercially accepted manner. The boxes of fruit were placed on a truck and hauled to the packing shed where a sub-sample of 20 fruits was taken from each box. The sub-sample was taken from top to bottom along the side of each harvested box. Fruits showing evidence of rot or other defects were avoided. Each fruit was placed in a depression of a new cardboard tray commonly used for tray-packing apples. The fruit was then placed in refrigerated storage overnight and cooled to a temperature of approximately 40° F, and removed to laboratory benches at room temperature for the remainder of the experiment. In this way an attempt was made to simulate the conditions encountered by peaches during the time from harvest to final sale to the consumer. However, these fruits received more gentle handling than they would have received in commercial channels. Counts of rotting and sound fruits were made 4, 8, and 12 days after harvest.

Table 2. The percentage of storage rots in Red Delicious and Golden Delicious apples in field boxes sprayed with different spray chemicals, after 2 1/2 months storage, 1957.

Box treatment	Per cent Rot[a]	
	Red Delicious	Golden Delicious
mercuric chloride AC[b]	7.4**	1.7**
cycloheximide AC	4.9**	1.1**
copper sulfate AC	4.3**	3.1**
captan AC	5.1**	1.3**
glyodin AC	3.0**	3.0**
maneb AC	7.5**	1.4**
zineb AC	3.2**	2.9**
check AC	14.5	7.8
check NC	15.7	5.2

[a]Based on observations made on 7,110 fruits.
[b]AC-Boxes artificially contaminated with spores before being sprayed with chemicals. NC-Boxes naturally contaminated.
**Significantly less than the check treatments at 1 percent level. According to analysis of variance applied to the angles corresponding to the percentage.

RESULTS

The percentage of rotted fruits found in the Delicious apples in 1957 is presented in Table 2. With the Red Delicious, all treatments were significantly better (odds 99:1) than either of the check treatments. There was as much rot in the naturally contaminated check as in the artificially contaminated check boxes. This provides evidence that untreated boxes have a sufficiently high level of contamination to be an important source of inoculum for storage rots. Also, with Golden Delicious, all treatments were better (odds 99:1) than either of the check treatments. In this case, however, the addition of inoculum increased the percentage rot (odds 99:1) in the artificially contaminated check over that found in the naturally contaminated check. The over-all incidence of rot in the Golden Delicious apples was less than that in the Red Delicious.

The percentage rot found in Romes and Staymans in 1958 is given in Table 3. In general, the results are similar to those found in 1957 on Red and Golden Delicious except that the captan spray and dip were relatively ineffective on Stayman and the zineb dip was ineffective on Rome.

Results of the experiments with peaches are presented in Table 4. It is apparent that the boxes used in the Kearneysville experiment carried a higher load of inoculum than those in the Romney experiment and, also, the fruits had a higher incidence of incipient infection than did those from Romney. The reduction in rot incidence due to treatment indicated by these experiments is very great.

DISCUSSION

These experiments, limited in scope, indicate that the savings made possible by box treatment may be of major importance. The commercial grower, fruit dealer, or processor who stores apples for extended periods of time or who handles peaches might all profit from its use. The cost of materials for treating the boxes ($0.25 for materials for 100 boxes) would be negligible when compared with the value of the fruit saved (at a valuation of $2.00 per box for the fruit, indications are that fruit worth a minimum of $10.00 to a maximum of

Table 3. The percentage of storage rots in Rome Beauty and Stayman Winesap apples in field boxes treated in 2 different ways with different spray chemicals, after 2 1/2 months storage, 1958.

Box Treatment	Per cent Rot[a]			
	Rome Beauty		Stayman Winesap	
	Sprayed	dipped	Sprayed	dipped
captan 2 lb./100 gal.	2.6**	1.2**	11.2	8.3**
zineb 2 lb./100 gal.	1.7**	7.1	7.3**	3.3**
amobam 1 qt./100 gal.	5.3*	1.4**	5.1**	3.3**
glyodin 1 qt./100 gal.	2.8**	3.9	5.4**	4.5**
new boxes, unsprayed	3.9**	-	6.4**	-
used boxes, unsprayed	7.5	6.1	12.1	14.0

[a]Based on observations made on 11,187 fruits.
*Significantly less than check treatment at 5 percent level, according to analysis of variance applied to the angles corresponding to the percentage.
**Significantly less than check treatment at 1 percent level.

Table 4. The percentage of post-harvest rots in Hale Haven peaches harvested in field boxes sprayed with different spray chemicals, 1958.

Box Treatment	Per cent rot[a] at two locations					
	Romney			Kearneysville		
	Days after harvest			Days after harvest		
	4	8	12	4	8	12
captan	1.0*	1.0*	12.1*	4.8**	23.0*	44.9**
zineb	0.3**	0.5*	3.6**	14.3	28.2	46.6*
sulfur[b]	0.3**	2.9*	6.9**	24.0	31.8	46.6*
amobam	1.0*	4.1*	7.8**	19.1	38.5	50.9*
cardboard liner[c]	0.0**	0.3*	1.0**	1.2**	5.6**	14.8**
untreated	7.7	17.8	44.9	25.8	47.0	74.8

[a]Based on observations made on 1,200 fruits.
[b]Five pounds actual sulfur in 100 gal. water. For other treatments see Table 3.
[c]"egg-shell" liners commonly used for tray packing apples.
*Significantly less than check treatment at 5 percent level, according to analysis of variance applied to the angles corresponding to the percentage.
**Significantly less than check treatment at 1 percent level.

$40.00 can be saved in each 100 boxes of fruit). It is likely that more efficient and perhaps more effective methods can be found for treating boxes. The list of chemicals tried is limited. Possibly there are other materials that would be equally or more effective than the ones tested. However, captan, glyodin, or sulfur can be used for box treatment without danger of violating the provisions of the Miller amendment, Public Law 518.

The results of these experiments support the hypothesis that field boxes serve as an important source of inoculum for storage rots of apple and post-harvest rots of peaches. The results in Table 4 on peaches leave little room for other interpretation. However, the results in Table 3 on apples do lend themselves to partial explanation in another manner. This is indicated by the incidence of rot found in the new unsprayed boxes. This, however, may be explained on the basis of a pathogenic spore load on the fruits when they were placed in the box. A difference in spore load (both qualitative and quantitative) on the fruits could also help explain the partial or total failure of some of the chemical treatments. However, it is apparent that this phase of fruit handling is of importance in preventing the development of post-harvest rots. The application of control measures should yield immediate practical benefits.

WEST VIRGINIA AGRICULTURAL EXPERIMENT STATION, MORGANTOWN

A PRELIMINARY REPORT ON THE COMPARATIVE EFFICACY OF COPPER-LIME AND AGRI-MYCIN DUST MIXTURES FOR THE CONTROL OF WALNUT BLIGHT IN OREGON[1, 2]

P. W. Miller[3]

The results of investigations carried on in Oregon from 1931 to 1948 (2, 3, 4) show that walnut blight can be controlled by the application of a sufficient number of certain dust mixtures. Under Oregon conditions, the best results have been obtained from the use of a dust mixture composed of 15 percent monohydrated copper sulfate, 30 percent hydrated lime, 10 percent dusting sulfur and 1 1/2 percent light mineral oil. In an epidemic blight year, six applications of this dust formulation applied at approximately weekly intervals beginning in the early pre-bloom stage are necessary for satisfactory control.

While this dust mixture has given relatively good control, it does cause limited foliage injury in the form of an interveinal spot necrosis under certain conditions. Moreover, the presence of dew or moisture on the foliage when the dusts are applied is necessary for the best results; otherwise, the dust mixture will be washed off by rain. There is need for an alternate efficacious dust mixture which would adhere better, cost less, and have a longer duration of effectiveness.

In 1956, Ark and Alcorn (1) reported that a 500 and 1000 ppm streptomycin-pyrophyllite dust mixture gave good control of walnut blight under California conditions, both giving somewhat better control than a 10 percent Copper A compound dust.

Investigations of the control of walnut blight in Oregon by dusting with antibiotic dust formulations were begun in Oregon in 1957. The results of field tests carried on in 1957 and 1958 are reported herein.

MATERIALS AND METHODS

In 1957, an Agri-mycin dust mixture containing 0.05 percent streptomycin, 0.005 percent oxytetracycline and 99.945 percent inert materials, and an Agri-mycin-copper dust containing 0.05 percent streptomycin, 0.005 percent oxytetracycline, 2.5 percent copper (metallic) and 97.445 percent inert materials were tested comparatively with a copper + lime + sulfur + oil (15-30-10-1.5) dust mixture in 50-tree plots in a Franquette orchard near Salem, Oregon.

The dust mixtures tested in 1958 were: 1) an Agri-mycin-copper dust containing 0.05 percent streptomycin, 0.005 percent oxytetracycline, 2.5 percent copper (metallic) and 97.445 percent inert materials, and 2) a copper + lime + sulfur + oil (15-30-10-1.5) dust.

Four dust treatments applied at approximately 10-day intervals beginning in the early pre-bloom stage were made each season.

RESULTS

In 1957, the Agri-mycin-copper dust mixture apparently was more effective than Agri-mycin dust without copper, giving practically as good control as the copper-lime dust mixture (Table 1). However, the Agri-mycin-copper dust caused more foliage injury under the conditions prevailing in 1957. The copper present in the mixture apparently was responsible for the foliage injury, as no visible injury occurred in the plot that was treated with Agri-mycin dust containing no copper.

In 1958, the Agri-mycin-copper dust mixture again gave relatively good control of the disease, even better than copper-lime dust. Under the environmental conditions prevailing in 1958 the Agri-mycin-copper dust mixture caused no visible foliage injury.

While additional tests are necessary before this antibiotic-copper dust mixture can be recommended unreservedly, the results are sufficiently promising to warrant limited trial by growers who dust for the control of walnut blight. With respect to residue tolerance, the

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[1]Cooperative investigations by Crops Research Division, Agricultural Research Service, United States Department of Agriculture, and Oregon Agricultural Experiment Station.

[2]Acknowledgment is made to Chas. Pfizer and Co., Inc., for donating Agri-mycin dust mixtures for test.

[3]Plant Pathologist, Crops Research Division, Agricultural Research Service, United States Department of Agriculture.

United States Food and Drug Administration has approved the use of Agri-mycin on Persian walnuts up to and including the early postbloom stage. The cost of Agri-mycin-copper dust mixture is less than of copper-lime dust, which is an additional factor in favor of its use.

Table 1. Comparative efficacy of Agri-mycin dusts and copper-lime dust mixtures for the control of walnut blight in Oregon, 1957-1958.

Year	Dust mixtures	Number of applications	Relative amount of foliage injury	Nuts examined (number)	Nuts infected (percent)
1957	Copper + lime + sulfur + oil dust[a]	4[b]	Trace	1100	10.9
	Agri-mycin-copper dust[c]	4	Moderate	799	11.0
	Agri-mycin dust[d]	4	None	1000	16.7
	Untreated	0	---	1000	30.1
1958	Agri-mycin-copper dust[c]	4[b]	None	2652	3.9
	Copper + lime + sulfur + oil dust[a]	4	Trace	2673	6.8
	Untreated	0	---	3257	25.7

[a]Monohydrated copper sulfate 15 percent, hydrated lime 30 percent, sulfur 10 percent, light mineral oil 1.5 percent, inert ingredients 43.5 percent.
[b]Applications made at approximately 10-day intervals beginning in the early prebloom stage.
[c]Streptomycin 0.05 percent, oxytetracycline 0.005 percent, copper (metallic) 2.5 percent, inert ingredients 97.445 percent.
[d]Streptomycin 0.05 percent, oxytetracycline 0.005 percent, inert ingredients 99.45 percent.

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A METHOD FOR MOUNTING ROOTS TO BE PHOTOGRAPHED

Donald V. McVey and J. W. Gerdemann[1]

Considerable difficulty is sometimes encountered in obtaining good photographs of diseased roots. When roots are lifted from water they mat together and usually appear like a "rat's tail." In addition, moist material produces undesirable reflections. When such specimens are allowed to dry, color differences and details are frequently lost. These problems can be eliminated if the material to be photographed is mounted in water From the appearance of published photographs of root systems, it is apparent that this technique has been used by a number of investigators and a similar technique has been described for photographing medical specimens[2]

When roots are placed in water horizontally between two pieces of good quality glass, they can be spread apart in one plane, and objectionable reflections eliminated. The mounted root is then raised above the background and the lights are adjusted to eliminate shadows. Roots mounted in such a way have a natural appearance, and small differences in color and detail are retained.

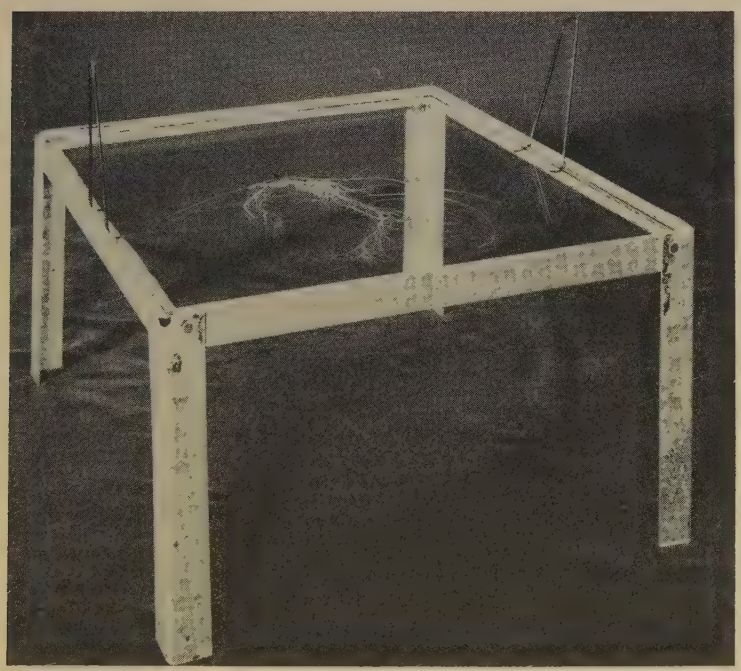

FIGURE 1. An apparatus for photographing roots mounted in water between two plates of glass.

The apparatus shown in Figure 1 consists of a frame made from sheet aluminum. A square is removed from the interior, leaving only the outer 1 1/2 inches. A 1-inch square is removed from each corner. The aluminum is then bent so that each side is L-shaped, 1 inch high and 1/2 inch wide. Legs are riveted to the corners of the frame. The 1/2 -inch lip is covered with caulking compound and a piece of flint plate glass inserted. The glass is pressed down and the excess caulking compound is removed. The corners are also filled with caulking compound. In using this apparatus a short piece of glass rod is placed vertically in each corner and enough water is added to bring it to the top of the glass rods. The roots are placed in the water and spread apart and the second flint plate glass is lowered into place with two pieces of stiff wire bent in the shape of a "U" with a prong on each end. Air bubbles that become trapped between the glass plates may be expelled by raising and slowly lowering one side of the plate of glass.

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[1] Respectively, Research Assistant and Associate Professor, Department of Plant Pathology, University of Illinois, Urbana, Illinois.

[2] Anonymous. 1958. The photography of gross specimens. Kodak Publication No. N-5. 24 pp.

ISOLATION OF RHIZOCTONIA SOLANI KUEHN FROM NATURALLY INFESTED AND ARTIFICIALLY INOCULATED SOILS

G. C. Papavizas and C. B. Davey[1]

Summary

Mature buckwheat (Fagopyrum esculentum) stem pieces buried for 4 days in naturally infested soils or soils inoculated with graded amounts of isolates of Rhizoctonia solani Kuehn were readily colonized by this fungus. Interference from actinomycetes and bacteria was eliminated when isolations of R. solani from colonized buckwheat stem pieces were made on water agar containing 50 ppm each of aureomycin hydrochloride, neomycin sulfate, and streptomycin sulfate. The buckwheat method of isolation gave more rapid results than the bean seedling method and provided a more reliable criterion of inoculum potential of R. solani in the soil than the infection index of susceptible bean seedlings.

INTRODUCTION

Several methods of isolating Rhizoctonia solani Kuehn from soil have been developed. Some of these are elaborate and utilize special perforated glass or plastic immersion tubes (2,3,11). Others involve direct isolation of R. solani from hyphae present among the heavier soil particles of the residue of soil suspensions (15), or indirect isolation through the use of susceptible crops planted in infested soils (5).

Although it is now well established that R. solani is able to make free and independent growth through unsterilized soil (1), very little is known about its ability to colonize various organic substrates in soil. The ability of the fungus to colonize maize kernels buried in soil for certain periods of time has been explored successfully for the isolation of the pathogen from soil (7, 9). Other organic substrates with lower levels of readily available nutrients than maize kernels might be more suitable for the separation of R. solani from associated soil microorganisms. Saprophytic colonization of various substrates has been used to isolate other soilborne phytopathogenic fungi from soil. The principles and methods involved have been reviewed by Garrett (6). The present experiments were carried out with the following objectives in mind: (a) to find a substrate more suitable than maize kernels for colonization by R. solani in soil, and (b) to develop a method of isolating Rhizoctonia through the use of this substrate.

MATERIALS AND METHODS

Four isolates[2] of R. solani were used in this study. The basal media used were: (a) potato-dextrose agar (PDA); (b) V-8 juice-dextrose-yeast extract agar (VDYA) containing per liter: 200 ml V-8 juice[3], 3 gm $CaCO_3$, 5 gm dextrose, 2 gm yeast extract, 20 gm agar, 800 ml distilled water (12); (c) VDYA containing 5 gm oxgall and 1 gm sodium propionate per liter (VDYA-5-1); (d) 2 percent water agar.

Fresh solutions of antibiotics[4] at 50 ppm each were added to the media after they had been melted and cooled to approximately 48° C. Media were delivered at the rate of 15 ml per plate with the aid of a Brewer automatic pipetting machine. Preliminary investigations with single antibiotics had demonstrated that aureomycin hydrochloride (A), albamycin sodium salt (Al), chloromycetin (C), neomycin sulfate (Ne), streptomycin sulfate (S) and Terramycin hydrochloride (T) at 50 ppm were able to inhibit bacterial growth without appreciably diminishing growth of R. solani. The following two combinations (50 ppm of each antibiotic) were used with each

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[1] Mycologist and soil scientist, respectively, Crops Research Division, Agricultural Research Service, United States Department of Agriculture.

[2] Isolate 5 was obtained from soil received from Seabrook Farms, Seabrook, N. J. Isolates 195, 353, and 361 were obtained through the courtesy of W. J. Zaumeyer.

[3] Trademark of a vegetable-juice product of the Campbell Soup Company.

[4] The antibiotics were obtained through the courtesy of the following: Lederle Laboratories (aureomycin hydrochloride), Parke, Davis & Co. (chloromycetin), Chas. Pfizer Co., Inc. (Terramycin hydrochloride) and the Upjohn Co. (neomycin sulfate and albamycin sodium salt).

of the four media: aureomycin hydrochloride + neomycin sulfate + streptomycin sulfate (A + Ne + S); albamycin sodium salt + chloromycetin + Terramycin hydrochloride (Al + C + T). The plates were inoculated in the center with 3-mm disks from the periphery of actively growing 3-day-old cultures of R. solani, and the rate of linear growth was measured daily on triplicate plates incubated at 26° C.

Internodal stem pieces 5 to 8 mm long of mature, dry buckwheat (Fagopyrum esculentum) were used to isolate R. solani from naturally infested or artificially inoculated soils. Attempts to increase the nutrient value of such pieces by steeping them in solutions of ammonium nitrate resulted in vigorous colonization of the pieces, when buried in soil, by Aspergillus niger v. Tiegh. and Trichoderma viride Pers. ex. Fr. In preliminary tests buckwheat was selected from a number of mature organic residues as the most suitable substrate for colonization by R. solani. Mature buckwheat stem pieces plated on acidified PDA and water agar before burial in soil yielded no Rhizoctonia.

Buckwheat stem pieces were mixed with Elsinboro sandy loam or a greenhouse loamy sand at the rate of 0.5 percent oven-dry weight. In other cases mature buckwheat stems 15 to 20 cm long were vertically inserted into the same soils. These soils had been passed through a 2-mm sieve and maintained at one-half of their moisture-holding capacity. Soil temperature in the greenhouse ranged from 22° to 26° C. Except when otherwise noted, the pieces were recovered after 4, 8, and 12 days, washed for 30 minutes in running tap water, surface sterilized for 1 minute with 1 percent sodium hypochlorite solution, and soaked for 16 hours at 5° C in sterile distilled water containing A + Ne + S (100 ppm of each). Five pieces were plated out on each of 10 Petri plates of water agar containing the same three antibiotics at 50 ppm each, and the plates were incubated at 26° C until positive identifications of R. solani could be made. Results were recorded as percent of pieces yielding R. solani on the test medium. Maize kernels subjected to the same series of treatments as the buckwheat stem pieces yielded high percentages of species of Mucor and Rhizopus but very little Rhizoctonia. Consequently, results obtained through their use have been omitted.

Isolations from 21-day-old Topcrop snap bean seedlings grown in the same soils mentioned earlier were made by cutting hypocotyls into pieces 5 to 8 mm long, treating these as the buckwheat pieces prior to plating, and plating out 50 pieces from each replication on the water agar medium. An infection index was derived by rating each surviving plant on an arbitrary scale of six degrees of infection (0 through 5) according to the extent of visible damage caused by R. solani.

RESULTS

Effect of Antibiotics on the Rate of Radial Growth of R. solani

The effect of the two combinations of antibiotics (50 ppm of each in the medium) on the rate of linear growth of four isolates of R. solani is shown in Table 1. The linear growth rate of all isolates was not affected by A + Ne + S incorporated with water agar or by either combination of antibiotics with VDYA or VDYA-5-1. The combination A + Ne + S reduced the linear growth rate of isolates 195 and 361 on PDA only. The linear growth rate of all isolates was reduced by Al + C + T in PDA and water agar and by oxgall and sodium propionate in VDYA-5-1.

Although colonies of all isolates were very thin and the total growth was much less on water agar (with or without antibiotics) than on the other media tested, the rate of linear extension of individual hyphae from the point where the inoculum was placed was very rapid. Hyphae of isolate 5, for instance, reached the edge of 90-mm Petri plates in 3 days. The fact that A + Ne + S allowed a rapid linear growth of representative isolates of R. solani on a minimal medium (water agar) was considered in the present tests a very desirable point. Except when noted, water agar with these three antibiotics at 50 ppm each was used hereafter for isolation purposes.

Isolation of R. solani from Naturally Infested Soil

Thirty gallons of a greenhouse loamy sand with low inoculum potential of R. solani was divided into two 15-gallon portions. Eight consecutive plantings of susceptible snap beans in one portion, plowed under at 3-week intervals, increased the inoculum potential to such an extent that in the last two plantings 95 to 97 percent of the seedlings were heavily infected. Each of the two portions was divided into five 3-gallon aliquots, placed in tared 3-gallon glazed crocks, planted with 20 bean seeds, and maintained in the greenhouse. The infection index of bean seedlings and the percent isolation of R. solani by means of buckwheat stems inserted

vertically in the same infested and noninfested soils immediately after seedlings had been removed are recorded in Table 2. Although an infection index of 5.0 (the highest on the scale) was recorded in all five replications of beans grown in the thoroughly infested soil, percent of buckwheat pieces yielding R. solani did not exceed 45. This was attributed to the "masking" effect of rapidly growing species of Mucor, Rhizopus, and Trichoderma. These fungi were favored by the long incubation of stems in the soil (1 and 3 weeks) and by the use of VDYA instead of the usual water agar medium.

Table 1. Effect of two combinations of antibiotics[a] on the rate of linear growth[b] of four isolates of R. solani on four media.

Medium	Isolate of R. solani			
	5	195	353	361
Potato-dextrose-agar				
No antibiotics	11.3	4.5	4.0	4.8
A + Ne + S	11.3	2.8	3.8	2.5
Al + C + T	10.7	3.4	3.4	3.5
V-8 juice-dextrose-yeast extract agar (VDYA)				
No antibiotics	11.1	3.0	4.5	5.3
A + Ne + S	11.3	2.8	4.9	5.0
Al + C + T	11.0	3.2	4.8	5.1
VDYA + 5 gm oxgall + 1 gm sodium propionate/liter				
No antibiotics	3.8	1.8	2.3	1.8
A + Ne + S	5.3	1.5	2.3	1.9
Al + C + T	3.8	1.5	2.2	1.9
Water agar				
No antibiotics	10.6	2.2	4.5	4.6
A + Ne + S	10.1	2.8	4.7	4.4
Al + C + T	8.1	2.0	3.8	3.5

[a]A = aureomycin hydrochloride, Al = albamycin sodium salt, C = chloromycetin, N = neomycin sulfate, S = streptomycin sulfate, T = Terramycin hydrochloride.
[b]Millimeters per day based on the average of six radial measurements of triplicate colonies incubated for 4 days at 26° C.

Table 2. Infection index of 21-day-old seedlings of snap beans and percent isolation of R. solani from buckwheat stems buried for 1 and 3 weeks in the same soil subsequent to seedling removal.

Inoculum potential of R. solani in soil	Infection index[a]	Percent[b] isolation of R. solani from buckwheat stems buried in the soil.	
		1 week	3 weeks
Low	0.3	0	0
High	5.0	31	45

[a]Mean severity rating on surviving individual plants on a scale of 0 (no disease) to 5 (hypocotyls completely girdled).
[b]Average of five replications.

Isolation of R. solani from Mixtures of Infested with Noninfested Soil

Elsinboro sandy loam with very high inoculum potential of R. solani was mixed with Elsinboro sandy loam with very low inoculum potential at the following ratios: 1:0 (highly infested control soil), 1:1, 1:3, 1:7, 1:15, 1:31, 1:63, and 0:1 (control soil with low infestation). From

each mixture, six 6-inch unglazed pots were planted with 10 seeds of Topcrop snap beans and six 4-inch unglazed pots were used to incubate mature buckwheat stem pieces for 4 and 8 days. The infection index of 21-day-old bean seedlings, the percent isolation of R. solani from the seedlings, and the percent isolation from buckwheat pieces buried for 4 days in the 4-inch pots filled with the same mixtures are given in Table 3. All results on isolation from bean seedlings and from buckwheat stem pieces were obtained on the water agar medium 24 to 48 hours after plating out the pieces.

Table 3. Infection index and percent isolation of R. solani from hypocotyls of beans grown in mixtures of infested with noninfested soil, and percent isolation of the fungus from buckwheat stem pieces buried for 4 days in the same mixtures in separate containers.

Ratio of infested to noninfested soil	Infection index[a]	Percent[b] isolation of R. solani: Bean hypocotyls	Buckwheat stem pieces
1:0	1.6	56	71
1:1	2.5	41	47
1:3	3.0	51	42
1:7	1.4	32	38
1:15	1.4	20	39
1:31	1.8	19	21
1:63	0.7	8	9
0:1	0.2	0	1
LSD (5%)	0.7	5	7

[a]Mean severity rating on surviving individual plants on a scale of 0 (no disease) to 5 (hypocotyls completely girdled).
[b]Average of six replications.

The percent isolation of R. solani from buckwheat stem pieces and from bean hypocotyls became higher as the ratio of infested to noninfested soil increased. The increments were more regular with the buckwheat pieces than with bean hypocotyls. The infection index, however, was higher when beans were grown in the 1:1 and 1:3 mixtures than when they were grown in all other mixtures. Evidently the infection index is not always reliable for detecting differences in soil infestation by the pathogen. No sufficient differentiation of soil infestation among mixtures 1:1, 1:3, and 1:7 was obtained with the three methods used.

Fifty transfers to PDA slants were made from 50 colonized buckwheat stem pieces cultured for 24 hours on the water agar medium. Forty-six yielded pure cultures of R. solani, two yielded bacteria presumably resistant to or tolerant of the three antibiotics (A + Ne + S), and two yielded species of Rhizopus. Approximately 60 percent of the cultures of R. solani isolated on the PDA slants were pathogenic to beans.

Isolation of R. solani from Artificially Inoculated Soil

Three isolates of R. solani were grown separately in 300 ml wide-mouth jars on a sterilized mixture of vermiculite and maize-meal (90 percent vermiculite, 10 percent maize-meal, water to 20 percent moisture, w/v). After incubation for 16 days the contents of the jars of each isolate were mixed together. The following proportions by volume of inoculum to noninfested unsterilized Elsinboro sandy loam were prepared: 30:70, 10:90, 5:95, 2:98, and 0:100 (control soil). Three replicates were used in 1/2-gallon crocks for the pathogenicity test and isolation from seedlings and three replicates in 4-inch pots were used for the buckwheat test. With only two exceptions, the results given in Table 4 show a higher percent isolation of R. solani by means of buckwheat stem pieces buried for 4 days than by the seedling infection method. The buckwheat test was more sensitive in recovering the fungus from the inoculated soil than the seedling infection test. For instance, in the 2:98 mixture isolates 5, 353, and 361 were recovered from 87, 9, and 28 percent of the buckwheat pieces, respectively, whereas they were recovered only from 8, 0, and 3 percent of bean hypocotyls. The mixture containing the

highest concentration of inoculum (30:70) did not result in the highest infection index with all isolates used. Perhaps the high concentration of maize-meal added with the inoculum induced rapid multiplication of microorganisms antagonistic to R. solani in the soil. Sanford (13) reported reduction of the persistence of the pathogen in natural black loam supplemented with maize-meal.

Table 4. Infection index and percent isolation of R. solani from hypocotyls of snap beans grown in soil inoculated with graded amounts of three isolates of the pathogen, and percent isolation of the fungus from buckwheat stem pieces buried for 4 days in the same mixtures in different containers.

Inoculum: soil ratio	Infection index[a]	Percent[b] isolation of R. solani: Bean hypocotyls	Buckwheat stem pieces
	Isolate 5		
30:70	1.5	29	100
10:90	1.5	16	99
5:95	1.7	25	93
2:98	1.3	8	87
0:100	0.5	0	5
	Isolate 353		
30:70	1.7	21	25
10:90	2.9	24	7
5:95	2.7	40	8
2:98	1.9	0	9
0:100	0.5	0	5
	Isolate 361		
30:70	2.5	29	52
10:90	3.0	8	36
5:95	2.9	12	37
2:98	0.8	3	28
0:100	0.4	0	4

[a]Mean severity rating on surviving individual plants on a scale of 0 (no disease) to 5 (hypocotyls completely girdled).
[b]Average of three replications.

Effect of Incubation Period of Buckwheat Stem Pieces in Soil on the Percent Isolation of R. solani in vitro

Results on percent isolation of R. solani as affected by the length of incubation of buckwheat stem pieces in the soil are presented in Table 5. In all cases stem pieces or entire stems incubated for 4 days in the three inoculum: soil mixtures tested yielded the highest percentage of R. solani and those incubated for 12 days the smallest. The importance of the length of incubation in the soil of maize kernels to trap various soil-borne pathogenic fungi has recently been emphasized (7). Evidently R. solani is an early and vigorous colonizer of suitable organic tissues, but it is rapidly succeeded or masked by other species.

DISCUSSION

Antibiotics and other antimicrobial agents have been used to facilitate enumeration and isolation of soil fungi with the dilution-plate method (4, 8, 10). Their use in isolating soil-borne phytopathogenic fungi has been suggested (6, 14), but it has not been fully advanced. In the present investigation incorporation of three broadspectrum antibiotics (aureomycin hydrochloride, neomycin sulfate, streptomycin sulfate) with water agar allowed rapid linear growth of R. solani isolates without changing the morphological characters of the mycelium, suppressed bacterial growth, and reduced the growth and sporulation of rapidly growing soil saprophytes.

Table 5. Percent isolation of R. solani from buckwheat stems buried for 4, 8, and 12 days in artificially inoculated Elsinboro sandy loam.

Inoculum[a]: soil ratio	Percent[b] isolation of R. solani: Days entire stems buried vertically in inoculated soil			Days stem pieces buried in inoculated soil		
	4	8	12	4	8	12
30:70	90	56	1	80	64	7
10:90	97	40	10	78	64	54
0:100	1	0	1	1	0	1

[a]Isolate 5 was used.
[b]Average of four replications.

These attributes of the water agar medium were considered desirable for the separation of R. solani from associated microorganisms.

Positive identifications of R. solani could be made by placing an inoculated Petri plate on the stage of a compound microscope and examining the hyphae. Within 24 hours from the time of inoculation actively growing tips of typical Rhizoctonia hyphae could be seen 10 to 15 mm away from the inoculum or from colonized buckwheat pieces. These hyphae were free of bacteria and showed the typical morphology of the Rhizoctonia mycelium.

Even though the percentage of isolation of R. solani by the buckwheat method from naturally infested or from inoculated soils was not always higher than by the seedling infection method, the buckwheat method was simpler and more rapid. It required 4 days for incubation of buckwheat pieces in the soil and an additional day to identify or isolate the fungus from the water agar medium. The seedling infection method as used here required a total of 22 days. The infection index, on the other hand, was based only on visible symptoms whose expression depends on the isolate and the kind of host (5). It can be seen in Table 3 that the infection index was higher with beans grown in the 1:3 mixture than with those grown in the original infested undiluted soil (1:0). Moreover, the infection index as used here did not take into account preemergence damping-off caused by Rhizoctonia species and by a number of other soil-borne pathogens.

Selectivity is an undesirable point of the seedling infection method of isolation of R. solani from soil. It is partly overcome by planting several susceptible host plants in the soil in order to obtain a wide range of isolates (5). The degree of non-selectivity of the buckwheat method remains to be investigated. Although the buckwheat method provided a better understanding of the inoculum potential of R. solani than did either the seedling infection method or the infection index, no attempts were made to develop a quantitative method of estimating R. solani in the soil.

The buckwheat method of isolation was based on the ability of R. solani to colonize mature pieces of buckwheat stems buried in soil. Other organic substrates (alfalfa stems, barley straw, maize kernels, oak sawdust) were also colonized by the fungus in the soil. The sequence of colonization, the range of organic residues that can be colonized competitively by R. solani, and the factors affecting colonization in unsterilized soils offer much scope for speculation and experiment.

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OBSERVATIONS OF THE LIFE CYCLES OF SOME WEST AMERICAN RUST FUNGI[1]

George B. Cummins[2]

A study of the western rust fungus flora was conducted in Montana, Colorado, and Arizona in the summers of 1955, 1956 and 1957, respectively. Particular attention was given to determining, by field observation, the life cycles and alternate host relationships of heteroecious species. In most instances observations started with the aecial stages in late June and early July and, frequently, the overwintered telia that were responsible for the aecial infections could be found closely associated. Specific sites were revisited, often repeatedly, to permit observation of the development of the uredial and telial stages during the summer and fall. When adequate isolation was attainable, field inoculation was attempted. In several instances, life cycle observations were verified by inoculations conducted in the Purdue University greenhouses with fungus and host materials collected in the West.

Under the relatively arid climate of the States concerned and if repeated visits are made to specific sites it is possible to determine the life cycles with a high degree of accuracy. Distance spread is limited not only by lack of conditions suitable for infection but also by the small and scattered population of the host plants. The hosts of obligately heteroecious species must be closely associated if the fungus is to persist in an area. Following are illustrating examples that were verified later by inoculations in the greenhouse.

1) In the Camus Prairie area of Flathead County, Montana, Puccinia recondita Rob. ex Desm. produced aecia on Clematis ligusticifolia Nutt. and uredia and telia on Elymus cinereus Scribn. & Merr. Heavy rusting occurred only when the Clematis and Elymus were intergrown; slight or no infection of the Elymus occurred if it grew 10 feet distant.

2) On a desert hillside near Paradise, Cochise County, Arizona, plants of Fouquieria splendens Engelm. (ocotilla) bore abundant Aecidium cannonii Griff. It was found that the aecia occurred only on those ocotilla plants whose base was intergrown with Bouteloua curtipendula (Michx.) Torr. (sideoats gramma) bearing telia of Puccinia vexans Farl. This fungus, because of its amphispores, can persist on the sideoats gramma without utilizing its aecial host. This accounts for its occurrence northward to Wyoming and Indiana, whereas ocotilla occurs only from western Texas to southeastern California in the United States.

3) Puccinia conspicua Mains was believed previously to alternate only between Helenium hoopesii Gray, as the aecial host, and Koeleria cristata (L.) Pers. (Junegrass) as the telial host. In the high meadows of the Chiricahua Mountains, Cochise County, Arizona, the aecial stage was ubiquitous but Junegrass was rare and uninfected. Observations, made repeatedly from late June to early September, indicated that the telial stage developed on Agrostis scabra Willd. Inoculation provided verification. In the Pinaleno and Santa Catalina Mountains field evidence was equally convincing that Festuca arizonica Vasey also served as a telial host.

4) Puccinia redfieldiae Tracy on Redfieldia flexuosa (Thurb.) Vasey (blowout grass) occurs in the sandhill regions of the western Great Plains. Aecidium anograe Arth. also occurs on Oenothera nuttallii Sweet in similar ecological situations. Dr. John W. Baxter suggested that A. anograe might be the aecial stage of P. redfieldiae. In August 1956 rusted blowout grass was located near Roggen and Sterling, Colorado. These specific sites were examined in June 1957 and the aecial stage was abundant on the evening primrose. In September 1957 the telia were collected and were used successfully to produce the aecial stage on O. nuttallii at Lafayette, Indiana in the spring of 1958.

The observed life cycles and alternate host plants, where field evidence was convincing, are summarized in Table 1. When a species name is followed by F or G the life cycle was verified by field or greenhouse inoculation, respectively.

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Table 1. A summary of the life cycles of western rust fungi.

Rust fungus	Telial host	Aecial host	State
Coleosporium jonesii	Ribes cereum	Pinus edulis	Ariz.
C. asterum	Aster conspicuus	P. contorta	Mont.
Cronartium coleosporioides (F)	Castilleja miniata	P. contorta	Mont.
C. coleosporioides	C. linariaefolia	P. ponderosa	Ariz.
C. coleosporioides	C. patriotica	P. ponderosa	Ariz.
C. comandrae (F)	Comandra pallida	P. ponderosa	Mont.
C. conigenum	Quercus grisea	P. chihuahuana	Ariz.
Cumminsiella mirabilissima (F)	Berberis repens	Berberis repens	Mont.
Gymnosporangium nelsonii	Juniperus scopulorum	Amelanchior alnifolia	Mont.
G. nelsonii	J. monosperma	A. utahensis	Ariz.
G. speciosum	Juniperus deppeana	Fendlera rupicola	Ariz.
Puccinia andropogonis	Andropogon scoparius	Pentstemon alpinus	N. Mex.
P. andropogonis	A. hallii	Petalostemon purpurens	N. Mex.
P. canaliculata	Cyperus rusbyi	Heliopsis parvifolia	Ariz.
P. caricina	Carex rostrata	Urtica dioica	Colo.
Puccinia conspicua (G)	Agrostis scabra	Helenium hoopesii	Ariz.
P. conspicua	Festuca arizonica	H. hoopesii	Ariz.
P. coronata	Calamagrostis canadensis	Rhamnus alnifolia	Mont.
P. coronata	C. inexpansa	Shepherdia canadensis	Colo.
P. coronata	C. purpurascens	S. canadensis	Colo., Mont.
P. coronata	C. rubescens	S. canadensis	Mont.
P. crandallii	Festuca idahoensis	Symphoricarpos occidentalis	Mont.
P. crandallii	F. ovina	S. occidentalis	Mont.
P. crandallii	F. scabrella	S. occidentalis	Mont.
P. crandallii	Hesperochloa kingii	S. albus	Colo.
P. crandallii	Poa fendleriana	S. albus	Colo.
P. crandallii	P. fendleriana	S. oreophila	Ariz.
P. dioicae	Carex filifolia	Agoseris glauca	Mont.
P. dioicae	C. praegracilis	Solidago missouriensis	Ariz.
P. dioicae	C. rusbyi	Artemisia caruthii	Ariz.
P. liatridis	Koeleria cristata	Brickelia grandiflora	Ariz., Colo.
P. monoica	K. cristata	Arabis drummondii	Colo.
P. monoica	K. cristata	A. holboellia	Mont.
Puccinia pygmaea	Koeleria cristata	Berberis repens	Ariz., Colo.
P. recondita	Agropyron dasystachyum	Anemone globosa	Mont.
P. recondita	A. griffithsii	Delphinium sp.	Colo.
P. recondita	A. latiglume	Aquilegia formosa	Mont.
P. recondita	A. latiglume	A. coerulea	Colo.
P. recondita	A. latiglume	Anemone globosa	Colo.
P. recondita	A. spicatum	Clematis hirsutissima	Colo.
P. recondita (F)	A. spicatum	C. ligusticifolia	Mont.
P. recondita	A. subsecundum	Thalictrum fendleri	Colo.
P. recondita	A. subsecundum	Phacelia heterophylla	Mont.
P. recondita	A. trachycaulum	Clematis hirsutissima	Colo.
P. recondita (G)	A. trachycaulum	C. ligusticifolia	Colo.
P. recondita	A. trachycaulum	Delphinium geyeri	Colo.
P. recondita	A. trachycaulum	D. occidentale	Colo.
P. recondita	A. trachycaulum	Hydrophyllum fendleri	Colo.
P. recondita	A. trachycaulum	Phacelia heterophylla	Ariz.
P. recondita (G)	A. trachycaulum	Thalictrum fendleri	Colo.
Puccinia recondita	Bromus anomalus	T. fendleri	Colo.
P. recondita	B. ciliatus	Thalictrum fendleri	Colo.
P. recondita	B. marginatus	T. fendleri	Colo.
P. recondita	B. purgans	Phacelia heterophylla	Colo.
P. recondita (F)	B. vulgaris	Thalictrum occidentale	Mont.
P. recondita	Elymus ambiguus	Clematis ligusticifolia	Colo.
P. recondita (G)	E. canadensis	C. ligusticifolia	Colo.
P. recondita (G)	E. cinereus	C. ligusticifolia	Colo., Mont.
P. recondita	E. glaucus	Hydrophyllum fendleri	Colo.
P. recondita (G)	E. glaucus	Thalictrum fendleri	Colo.
P. recondita (G)	E. glaucus	T. occidentale	Colo., Mont.
P. recondita	Festuca thurberi	T. fendleri	Colo.
P. recondita	Sitanion hystrix	Phacelia heterophylla	Ariz.
P. recondita	Trisetum spicatum	Thalictrum fendleri	Colo., Mont.
P. redfieldiae (G)	Redfieldia flexuosa	Oenothera nuttallii	Colo.
P. stipae	Stipa comata	Chrysopsis villosa	Ariz., Mont.
P. stipae	S. comata	Chrysothamnus sp.	Colo.
P. stipae (F)	S. comata	Erigeron corymbosus	Mont.
P. stipae	S. comata	Grindelia squarrosa	Colo.
P. stipae	S. comata	Gutierezia sarothrae	Colo.
P. stipae	S. comata	Lygodesmia juncea	Colo.
P. stipae	S. comata	Solidago missouriensis	Ariz., Colo.
P. vexans (F, G)	Bouteloua curtipendula	Fouquieria splendens	Ariz.
Uredinopsis pteridis	Pteridium aquilinum	Abies grandis	Mont.

RESISTANCE OF STRAWBERRY VARIETIES AND SELECTIONS TO LEAF SPOT AND SCORCH[1]

Jules Janick and E. B. Williams

Abstract

A total of 41 strawberry varieties and selections were sampled in replicated trials for incidence of leaf spot, caused by Mycosphaerella fragariae, and leaf scorch, caused by Diplocarpon earliana. Of the varieties tested, Aroma, British Sovereign, Catskill, Crimson Flash, Empire, Fairfax, and Midland were found to exhibit the lowest incidence of infection when both diseases were considered.

INTRODUCTION

Leaf spot, caused by Mycosphaerella fragariae, and leaf scorch, caused by Diplocarpon earliana, are serious diseases of strawberries in Indiana, as well as in other strawberry-producing areas of the United States. Tremendous losses in Tennessee, Kentucky, and Arkansas resulted from an epidemic involving a complex of these diseases in 1957. The need for varietal evaluation of disease resistance to these diseases is everpresent, as the varietal picture in all areas is constantly changing. This has been especially true in recent years owing to the introduction of virus-free forms of some of the older varieties. In addition, the vigorous breeding programs in progress have introduced many outstanding new varieties, some of which appear extremely susceptible to these diseases (2). Accurate appraisals of resistance are especially valuable for breeding purposes as many of the older classifications listing varieties as either susceptible or resistant appear to be inadequate. These factors led to an attempt at an objective evaluation of the reaction of varieties and selections to these diseases.

MATERIALS AND METHODS

Three plantings (1955, 1956, and 1957) of strawberries, each containing a number of varieties, were evaluated in 1958 at the O'Neall Farm, Lafayette, Indiana. The trials received no fungicide applications. The 1955 planting consisted of 16-foot plots of five varieties of ordinary and virus-free plants replicated five times. Five additional varieties (Bellmar, British Sovereign, Catskill, Crimson Flash, and Wisconsin 214) were replicated twice in the guard rows. The 1956 and 1957 plantings contained 10-foot plots of 25 varieties or selections replicated six times. There were six plots each of three varieties (Aroma, Jumbo, and Redstar) in the guard rows of the 1956 plantings. Six plots of Fairfax were grown adjacent to the 1957 plantings.

The disease rating was made on leaf samples collected on June 26. A 1-foot square frame was placed at random in the center portion of the plot and all the leaves in the frame were collected into a paper bag, which was subsequently placed in a polyethylene liner and kept in cold storage until the samples were classified. Each sample of leaves, ranging in number from 8 to 34, was scored individually for both spot and scorch. A modification of Spangelo's (5) method for scoring was used. Six classes (1 through 6) were set up where 1 = no infection, 2 = 1 to 10 lesions per leaf, 3 = 11 to 20 lesions, 4 = 21 to 40 lesions, 5 = 41 to 60 lesions, and 6 = 61 and over lesions per leaf. The index was calculated by multiplying the number of plants in each class by the class number, adding the totals, and dividing by the total number of leaves.

The analysis of variance was calculated on the average score for each plot. The varieties in the guard rows were not incorporated in the analysis but are presented with the data.

RESULTS

The average score for leaf spot and scorch of each variety is presented in Table 1. The scores of the varieties present in the guard rows are in parentheses.

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Table 1. Reaction of strawberry varieties and selections to leaf spot and leaf scorch. Scale from 1.0 (no infection) to 6.0 (severe infection).

Variety or selection	Leaf spot class			Leaf scorch class		
	Year of planting					
	1955	1956	1957	1955	1956	1957
Armore	2.6		2.4	2.1		1.5
Aroma		(1.5)			(1.1)	
Bellmar	(1.3)	3.0		(4.0)	2.6	
Blakemore	2.5	3.6	2.6	1.1	1.1	1.2
Brit. Sovereign	(1.3)			(1.2)		
Catskill	(1.7)	2.3	1.4	(1.2)	1.1	1.1
Crimson Flash	(1.6)			(1.0)		
Dixieland		3.2	1.4		1.2	2.6
Dunlap		2.8			1.1	
Earlidawn		3.8	1.7		1.3	2.8
Empire		2.3	1.4		1.0	1.0
Erie			2.5			1.4
Fairfax			(1.8)			(1.2)
Jerseybelle			2.3			2.3
Jumbo		(3.0)			(1.2)	
Midland		2.4			1.2	
Pocahontas		3.0	1.8		1.5	3.7
Premier	1.8	2.5	2.0	1.1	1.3	1.4
Redglow		4.0	3.4		1.5	2.2
Redstar		(2.8)			(1.0)	
Robinson		3.2			1.2	
Sparkle	2.3	2.8	1.4	1.6	1.3	1.7
Stelemaster		3.5			1.2	
Surecrop		2.0	2.0		1.5	2.0
Tennessee Beauty	1.6	2.3	1.6	1.6	1.3	1.4
Tennessee Shipper		2.8			1.2	
Vermillion		1.9	1.9		1.5	1.6
Wisconsin 214	(1.8)			(1.0)		
Md. U.S. 2210		3.4			1.0	
U.S. 2359		3.5	1.7		1.1	2.0
Md. U.S. 2389			1.5			1.8
Md. U.S. 2519			3.5			1.8
Md. U.S. 2555			3.1			1.1
Md. U.S. 2601			3.1			1.2
Md. U.S. 2610			2.2			1.6
U.S. 3919		2.1	1.6		1.4	1.5
U.S. 3961		2.6	1.6		1.2	1.3
U.S. 3972		2.6			1.5	
U.S. 4152		2.4			2.4	
U.S. 4192		2.6	1.6		1.3	2.2
U.S. 4333			2.7			2.5
Average	2.1	2.8	2.1	1.5	1.3	1.8
L.S.D. .05	1.0	1.2	.7	.7	.5	.7

The varieties were significantly different in all experiments, at least at the 5 percent level. The data, however, were quite variable and inconsistent in some cases, partially as a result of the differences in the 1956 and 1957 plantings. In every case where the varieties were in common in the two plantings, the varieties in the 1956 plantings had the same or higher spot scores and the same or lower scorch scores. In varieties such as Pocahontas and Earlidawn there was a very definite reciprocal relationship between scorch and spot. The average scores of the 1955 planting were very similar to the 1957 plantings.

As the data for both spot and scorch are continuous, the classification of varieties into clear-cut classes is not possible. The differences in disease scores for each planting reduces the confidence in the scores for those varieties that were restricted to only one planting. These results indicate that the varieties with the most resistance to spot and scorch include Aroma, British Sovereign, Catskill, Crimson Flash[2], Empire, Fairfax, and Midland[2]. Premier, Surecrop, Tennessee Beauty, Vermillion, Md. U.S. 2389, U.S. 3919, and U.S. 4152 had low spot scores. There were many varieties with low scorch scores including Blakemore, Dunlap, Jumbo, Redstar, Robinson, Stelemaster, Tennessee Shipper, Wisconsin 214, Md. U.S. 2210, Md. U.S. 2555, and Md. U.S. 2601.

DISCUSSION

The results reported here are in disagreement with portions of some of the other reported classifications (1, 3). This may be due to errors in classification, differences in inoculum potential, or possible races of the pathogens (4). This points out the value of the inclusion of a set of selected varieties in each planting set out for evaluation of spot and scorch. Extreme susceptibility to leaf spot and scorch is present in Redglow and Bellmar, respectively. Blakemore appears to be differentially resistant to scorch and susceptible to spot. A clear-cut example of resistance to spot and susceptibility to scorch was not observed.

The large variability in results would indicate that the classification proposed by Spangelo may be unnecessarily subjective. The differences between classes may not be strictly additive, which seriously interferes with assumptions inherent in the analysis of variance. An improvement might consist of actual counting of the "spots" on the middle blade of the leaf. More investigation is needed to devise a more precise sampling technique. Although Spangelo has suggested that the maximum infected leaf of each plant be used for seedling evaluation, the average score was thought to give a more reliable estimate of resistance for plot work. A comparison of the two methods indicates a fairly good relationship between ranks, especially of the extreme types. There still remains a need for a more precise method that is relatively simple to perform.

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[2] Crimson Flash and Midland are probably synonymous.

FUSARIUM INFECTION OF SAFFLOWER ROOTS ON ACID SOIL

C. A. Thomas

Attempts to grow safflower, Carthamus tinctorius L., during the past 6 years at Beltsville, Maryland, have been successful on some field plots and have failed on others. Lateral and tap roots of seedling plants from plots on which safflower grew poorly were pruned severely (Fig. 1).

FIGURE 1. Roots of safflower seedlings showing effects of Fusarium infection, three on left, compared with healthy roots.

Root tips as well as areas of the epidermis and cortex were necrotic. Numerous fungi were isolated from such areas, but a form of Fusarium oxysporum was obtained most consistently.

Preliminary inoculation tests were made by placing agar blocks from Petri dish cultures of the isolated fungi in contact with roots of seedlings germinating on moist filter paper. Only the Fusarium oxysporum caused infection. Attempts to produce typical symptoms in the greenhouse were made by growing seedlings in steamed soil artificially infested with a spore suspension of this organism. These tests were successful only when fresh "wild"-type isolates were used to infest soils obtained from field plots in which the trouble occurred naturally. No infection was evident in these soils when steamed but not infested, or in steamed, artificially infested soils from other plots.

A marked difference in pH was found among soil samples taken from the field plots. Soils of three plots on which safflower grew poorly and showed root infection had a pH of 4.3, 4.7, and 5.0; whereas soils of three plots on which good growth and no infection occurred had pH values of 6.2, 6.8, and 7.2. Application of hydrated lime to the acid soils at rates of 1500 to 3000 pounds per acre gave complete control of the root infection in pot tests in the greenhouse and in field plots.

The Fusarium infection of safflower roots described here may result from the effects of soil acidity on growth of both the organism and suscept. Soil acidity is known to favor development of several biologic forms of F. oxysporum (1). Safflower is poorly adapted to acid soils. Calcium deficiency may be involved. Ranney and Bird (3) reported calcium effective in reducing seedling losses in cotton, presumably because of its beneficial effect (2) on the development of the cotton seedling.

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CRONARTIUM COMANDRAE IN WYOMING[1]

E. A. Andrews and M. D. Harrison[2]

The first report of Cronartium comandrae Peck on pine from Wyoming appears to be that of Hedgcock and Long (4) in 1915. They reported it on Pinus contorta Dougl., based on a collection made at Dubois by C. E. Taylor in 1914. Arthur (2) also lists it on this host for Wyoming.

During the past 25 years Dr. W. G. Solheim has collected the specimens of aecia on P. contorta listed in Table 1. Mielke (6) reports that in recent years the rust has been common

Table 1. Collections of Cronartium comandrae Pk. O, I on Pinus contorta included in the W. G. Solheim Herbarium.

Collection	Spore Stage	Year	Location
741	I	1934	Triangle X Ranch, Teton Co., Wyoming
859	I	1933	Porter Burn, Mountain Home, Medicine Bow Mts., Albany Co., Wyoming
4716	O & I	1957	East end of Biological Research Station pasture, Grand Teton National Park
4865	I	1957	8 miles east of junction of U. S. highways 89 and 26, Teton Co., Wyoming
4866	I	1957	8 miles east of junction of U. S. highways 89 and 26, Teton Co., Wyoming

and destructive in lodgepole stands over extensive areas of Wyoming. Also in 1957, aecial blisters were observed killing P. contorta in the Tongue and Buffalo districts of the Bighorn National Forest, and in the Clark Fork district of the Shoshoni National Forest (1).

Preliminary surveys during June and July of 1958 reveal the disease is widespread in Wyoming. Aecial blisters and spermagonia are prevalent on lodgepole in the Snowy Range of the Medicine Bow Mountains, near Lander[3] and Dubois in the Wind River Mountains, and near Dubois in the Absaroka Mountains. Uredia of C. comandrae on Comandra umbellata (L.) Nutt. have been found near infected pine at one or more sites in all three mountain ranges (Table 2).

The peridia of some of the aecial blisters were ruptured and the diagnostic, pyriform species were being liberated when first observed on June 4. It was not until about a week later that the pyriform spermatia present in the reddish droplets of ooze, described by Boyce (3), could be found. By June 24, a few Comandra plants within about 100 feet of infected pine could be found that were producing uredospores.

The aecial blisters ranged in size from recent, limited infections on small branches to cankers that extended 3 feet along the main trunk of dominant trees. The top one-half to two-thirds of many of them in each site had been killed by aecial cankers. Hedgcock and Long (4) have observed this disease killing 50 percent of Pinus pungens stands in Pennsylvania; Meinecke (5) found that the forest cover was reduced by 33 percent on study plots of P. ponderosa in California.

The extent to which this fungus has impaired the potential yield of timber in the Snowy Range forest area as a whole has not been determined. The disease was most severe at the

1 Published with approval of the Acting Director, Wyoming Agricultural Experiment Station, as Journal Paper No. 116.

2 Assistant Plant Pathologist and graduate assistant, respectively.

3 The same infected stands were observed in 1957 by Roger Peterson and are recorced in his unpublished field notes.

Table 2. Locations in Wyoming where aeciospores, spermatia, and uredospores of Cronartium comandrae Peck were collected from June 4 through July 3, 1958.

Mountain	County	Number of sites examined and the spore stages collected		
		Aeciospores	Spermatia	Uredospores
Snowy Range of the	Albany	6	2	3
Medicine Bow Mountains	Carbon	1	-	-
Wind River Mountains	Fremont	2	2	1
Absaroka Mountains	Fremont	1	1	1

edge of lodgepole stands adjacent to Comandra. Trees were large and widely spaced; reproduction was very sparse. About 150 feet in from the edge of the stand the thinning effect of the disease was not evident, the trees being crowded and uniformly small in diameter.

It is of interest that many of the severely infected stands of lodgepole occur near the lower limit of timber growth adjacent to sagebrush-comandra areas where resorts and recreational facilities are concentrated. These trees frequently have value in addition to their importance in water-shed protection and in timber production.

In order to determine more accurately the maximum distance that basidopsores of C. comandrae can effectively disseminate the disease in Wyoming, a more intensive study of a large number of infection sites will be necessary.

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BROWN SPOT NEEDLE BLIGHT ON EASTERN WHITE PINE

John S. Boyce, Jr.[1]

Partial dieback of current needles is one of the symptoms of white pine blight, a disease of unknown origin afflicting eastern white pine, Pinus strobus. Needle fungi are among the possible causes (3).

In describing the brown spot needle blight disease of pine seedlings caused by Scirrhia acicola (Dearn.) Siggers, P. strobus is listed as a host in North Carolina (2). However, this fungus has not been generally recognized as a cause of white pine needle blight.

Three collections of the brown spot fungus on blighted needles of eastern white pine were made in western North Carolina in 1957 and 1958. The first collection was made in a 30-year-old plantation in Yancey County in December 1957. At this time dieback of the 1957 needles was prevalent in the stand, and blighted needles were more abundant in the lower than in the upper crown. By the following July most of the infected needles had been shed.

A second occurrence was on naturally reproduced white pine, 15 to 60 feet tall, in Transylvania County near Brevard in April 1958. Most of the infected 1957 needles were still attached. A third collection was made in August 1958, about 1/2 mile away in a natural stand. Here the affected trees had largely shed the infected 1957 needles.

In each instance pycnidia of the brown spot fungus were present on the dead tips of the 1957 needles. A few needles were observed where pycnidia occurred on necrotic spots surrounded by green tissue. Single conidia taken from pycnidia were planted on malt agar. Typical cultures of S. acicola resulted which were identical with isolates previously obtained from loblolly (Pinus taeda) and longleaf pine (P. palustris) in Mississippi.

An additional record was made in June 1950, when it was noted that S. acicola pycnidia were present on the tips of 1949 needles that were shed prematurely near Arborvale, West Virginia.

It is not known whether needle blight caused by S. acicola is common on white pine, since no systematic search for it has been made. It is possible, however, that some occurrences of the so-called white pine blight disease in the Southeast, as described by Toole (3), are simply a result of needle infections by the brown spot fungus. It is worth noting that this fungus was not recognized as the cause of a widespread needle blight on loblolly pine until recently (1). Previously it was regarded as an important cause of needle blight only on longleaf pine seedlings.

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A NEW HOST OF SCLEROTINIA SCLEROTIORUM IN ARGENTINA

R. E. Pontis and J. M. Feldman[1]

Pink rot of celery (Apium graveolens), caused by Sclerotinia sclerotiorum (Lib.) d By., was found in celery fields, severely damaged, near Algarrobal, Las Heras, Provincia de Mendoza, in August 1958. The disease has occurred near harvest time and appeared as a basal rot of crown and petioles with black sclerotia. It has been artificially reproduced in the laboratory and in the field.

This appears to be the first time that S. sclerotiorum has been found on celery in Argentina. In this country, up to the present, this fungus has been determined on the following hosts:

1. Sunflower (Helianthus annuus)
2. Peanut (Arachis hypogaea)
3. Cruciferae, especially cabbage (Brassica sp.)
4. Potato (Solanum tuberosum)
5. Bean (Phaseolus sp.)
6. Dahlia (Dahlia variabilis)
7. Carrot (Daucus carota)
8. Lettuce (Lactuca sativa)
9. Broad bean (Vicia faba)
10. Tomato (Lycopersicon esculentum)
11. Citrus, especially lemon (Citrus sp.)

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POWDERY MILDEW OF APRICOT IN ARGENTINA

R. E. Pontis and J. M. Feldman[1]

During the spring of 1958, powdery mildew was observed on apricots of the collection of the Facultad de Ciencias Agrarias in Chacras de Coria, Lujan, Provincia de Mendoza.

The disease appears to comprise at least two distinct infections: a) fruit and leaf infection caused by conidia of Sphaerotheca pannosa Lév. produced on rose or peach, and b) leaf infection caused by Podosphaera tridactyla (Wallr.) d By., which multiplies principally or exclusively on apricot leaves[2]. Although this disease has been observed on peach and roses in this region for a long time, this is the first record of the disease on apricot in Argentine.

As the writers have not found perithecia and since powdery mildews cannot be adequately diagnosed on the basis of their imperfect stages, for the time being the causal fungus cannot be positively named.

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[1]Professor of Plant Pathology and Instructor in Plant Pathology, respectively.

[2]Yarwood, C. E. 1952. Apricot powdery mildew from rose and peach. California State Dept. of Agr. Bull. 41. pp. 19-25.

DOWNY MILDEW OF SUNFLOWER IN ARGENTINA

R. E. Pontis, J. M. Feldman, and A. Klingner[1]

During the spring of 1958, downy mildew (Plasmopara halstedii (Farl.) Berl. & de T.) was observed on sunflower (Helianthus annuus), cultivated as an ornamental on the campus of the Facultad de Ciencias Agrarias in Chacras de Coria, Lujan, Provincia de Mendoza. Microscopic examination showed the downy mildew fungus fruiting abundantly on the leaves. This is the first record of the disease in Argentina.

The fungus had already been registered in the following countries in 1954[2]: Brazil, Bulgaria, Canada, Chile, Dominican Republic, Rumania, Uganda, United States, U.S.S.R. and Yugoslavia.

Lately, Sackston[3, 4] pointed out downy mildew of sunflower for the first time in Chile in 1956, and in Uruguay in 1957, reporting that "...the disease is known in most sunflower-growing countries."

It is surprising that the determination for Argentine is being made for the first time in the Province of Mendoza, where the sunflower is not grown on a commercial scale. In the reviewed literature no references are found about the existence of this disease in the sunflower belt of this country.

It is almost certain that the seed comes from the sunflower belt, and that the fungus is carried with it. Consequently, it can be supposed that the disease, though not severe, exists in the above-mentioned region.

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[1]Professor of Plant Pathology, Instructor in Plant Pathology and Research Assistant, respectively.

[2]Commonwealth Mycological Institute. Distribution Maps of Plant Diseases. Map. No. 286. Revised to 1. iii. 1954.

[3]Sackston, W. E. 1956. Observations and speculations on Rust (Puccinia helianthi Schw.) and some other diseases of sunflowers in Chile. Plant Disease Reptr. 40: 744-747.

[4]Sackston, W. E. 1957. Diseases of sunflowers in Uruguay. Plant Disease Reptr. 41: 885-889.

NEW OR UNUSUAL RECORDS OF PLANT DISEASE OCCURRENCE

FUSARIUM EUMARTII WILT AND TUBER NECROSIS IN IDAHO

By James W. Guthrie[1]

Fusarium eumartii wilt of potatoes, caused by Fusarium solani (Mart.) Appel. & Wr. var. eumartii (Carpenter) Wr., is a common but not a serious disease in certain midwestern and eastern States, but has not been important in Idaho until recent years. In 1956 F. eumartii caused damage to a crop of Russet Burbank potatoes in a small certified seed-producing area in Idaho. In 1957 and 1958 this disease was found on more than 1000 acres of commercial potatoes and crop losses ranged from 5 to 100 percent. Stem-end tuber necrosis prior to shipment accounted for most of the loss (Fig. 1). In many instances detection of affected tubers was possible because of the presence of a bluish-grey color on the stem end. This condition is known to inspectors as "Blue Nose." The serious tuber rot stage called "Side rot" was of minor importance in Idaho. The field wilt symptom was often observed but losses due to wilting were not of major importance.

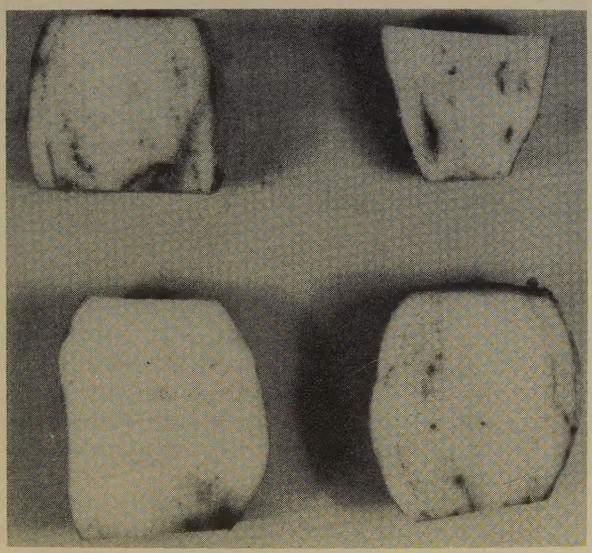

FIGURE 1. Fusarium eumartii induced tuber necrosis in the Russet Burbank variety.

Preliminary experimental tests for disease control conducted during 1958 on soil known to be infested with F. eumartii gave negative results. It was hoped that a chemical with systemic fungicidal properties would be found which would control this disease. None of the chemicals tested showed definite promise. As a preventative measure the Idaho Crop Improvement Association established a zero tolerance on F. eumartii wilt for potato seed certification.

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ACERIA TULIPAE K. FOUND FOR THE FIRST TIME IN IDAHO[1]

By Clarence J. Peterson, Jr. and J. M. Raeder[2]

The vector of wheat streak mosaic virus, Aceria tulipae K., was found for the first time in Idaho in September 8, 1958. The mite was found in Latah County in the vicinity of Moscow on wheat and orchard grass.

At the time the mite was found, many of the wheat plants in the area were infected with wheat streak mosaic virus and many of the leaves were rolled tightly along the midrib.

The mites were checked to determine if they were carrying the wheat streak mosaic virus by placing six mites per plant on six Idaed and seven Omar wheat plants in the laboratory. Five of the Idaed and four of the Omar plants developed mosaic symptoms within 10 days.

A survey of the surrounding counties failed to disclose the presence of Aceria tulipae K., however, three other Eriophyid species were found, as follows: 1) Siteroptes graminum Reuter (two spot mite) was found on wheat; 2) Vasates mckenzeiei K. was found on quack grass (Agropyron repens (L.) Beauv.); 3) Vasates dubius Nal. was found on Fescue (species undetermined). Dr. H. H. Keifer[3] of the California State Department of Agriculture, Sacramento, reported that this is the first time he has seen Vasates dubius in a North American collection.

IDAHO AGRICULTURAL EXPERIMENT STATION, MOSCOW, IDAHO

[1]Published with the approval of the Director, Idaho Agricultural Experiment Station, as Research Paper No. 461.

[2]Respectively, Graduate Student and Plant Pathologist, Idaho Agricultural Experiment Station.

[3]This information was obtained through personal correspondence with Dr. H. H. Keifer, who identified all the above species of mites.

SCAB DISEASE ON CANTALOUPES IN NORTH CAROLINA

By N. N. Winstead,
D. L. Strider,
and S. F. Jenkins

Plants of the Rio Gold and Hale's Jumbo varieties of cantaloupe severely infected with Cladosporium sp. were observed in an experimental planting at the Upper Mountain Research Station, Laurel Springs, North Carolina during the summer of 1957. Leaves, fruits, and stems were affected. Symptoms on the affected plant parts are shown in Figure 1.

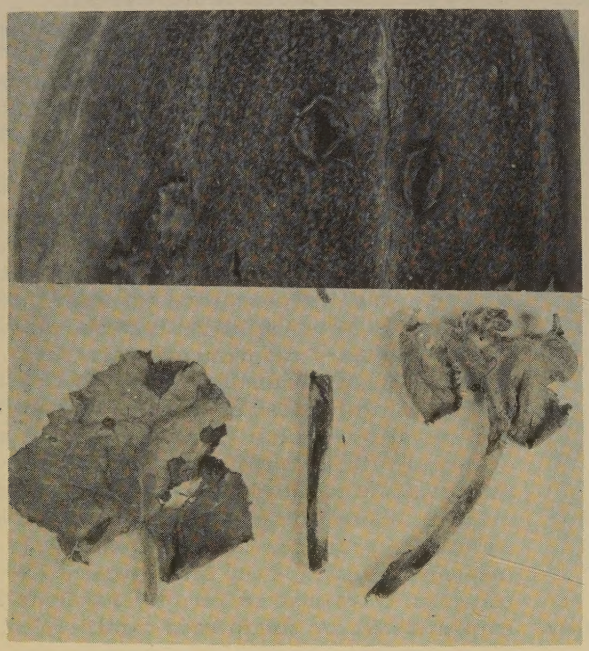

FIGURE 1. Close-up of a lesion on a cantaloupe fruit and infected leaves and stems.

The fungus isolated from cantaloupe fruits was found to be culturally identical with an isolate of Cladosporium cucumerinum Ell. & Arth., the causal agent of scab of cucumber. Inoculations were made on seedlings of the cucumber varieties Marketer and Fletcher[1] which are susceptible and resistant, respectively, to the scab disease and on seedlings of Rio Gold and Hale's Jumbo cantaloupes. The methods of inoculation used were similar to those described by Walker[2]. An isolate of the fungus from cantaloupe and an isolate of C. cucumerinum were used as inoculum.

Plants of the cantaloupe varieties Rio Gold and Hale's Jumbo, and of the cucumber variety Marketer, were highly susceptible to both isolates. Plants of the cucumber variety Fletcher were resistant to both isolates.

The scab disease is usually severe on squash and cucumber plantings in the mountain areas of North Carolina, however, this is the first report of its occurrence on cantaloupe in the field in North Carolina.

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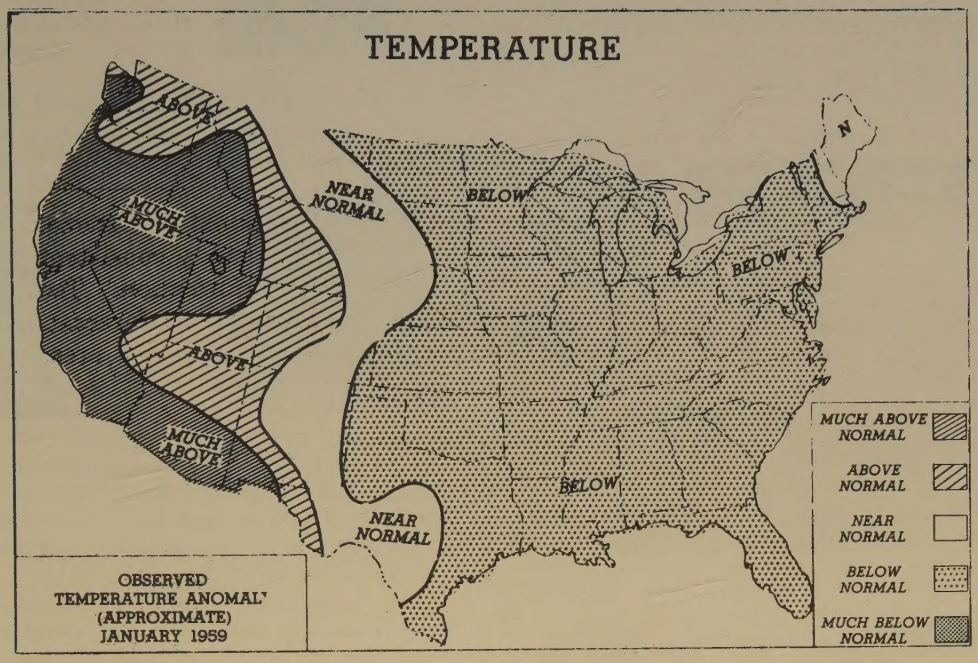
[1]Barham, W. S., and N. N. Winstead. Ashe and Fletcher, two new downy mildew and scab resistant cucumbers. North Carolina Agr. Exp. Sta. Bull. (In Press).

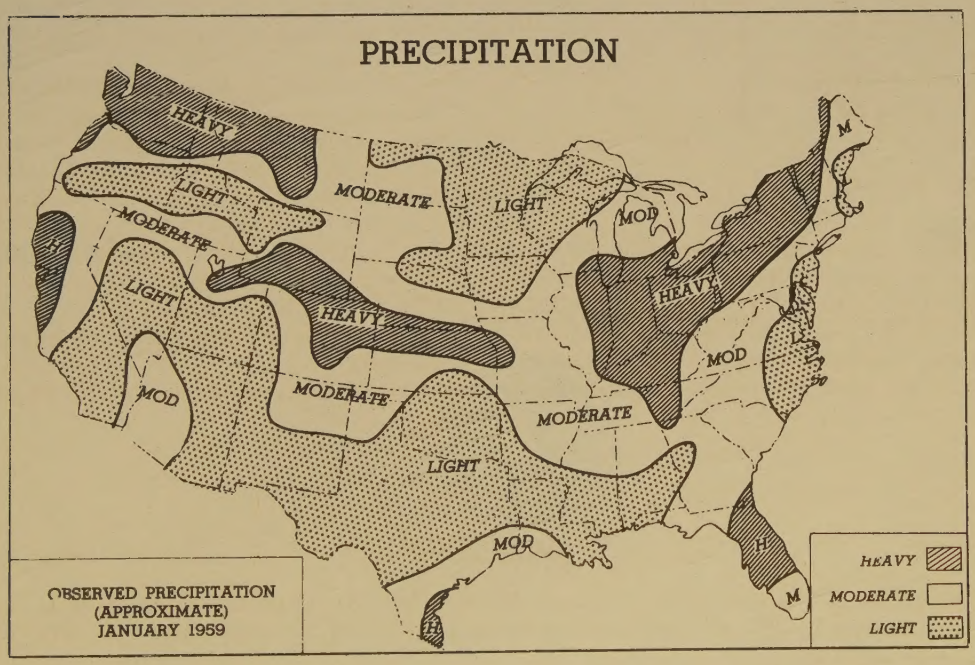
[2]Walker, J. C. 1950. Enivronment and host resistance in relation to cucumber scab. Phytopathology 40: 1094-1102.

CORRECTION

REPORTER, December issue (Volume 42, Number 12), page 1374: In Table 1, under the subtitle St. Joseph I, Y^{sc}/a^{a}, the values originally given by the authors are incorrect. The correction is as follows:

Instead of	Should read
476.5	1906
403.0	1612
453.8	1819
435.5	1742
495.5	1982
443.8	1775
416.5	1666
452.0	1808
430.3	1721
ns	ns
ns	ns





The terms used in the accompanying maps, which define the ranges of temperature and precipitation, are numerical class limits. These are based on a statistical analysis of past records through which is determined the normal frequency of occurrence of temperatures and precipitation at various times of the year for different locations. For temperature the classes above, below, and near normal are so defined that they each normally occur one-fourth of the time; much above and much below normal, one-eighth of the time. Precipitation is depicted in terms of light, moderate, and heavy, each class normally occurring one-third of the time and thereby having equal probability of occurrence. These maps graphically represent only the general trends and give the country's weather picture at a glance. For quantitative studies, where monthly mean temperatures and actual precipitation records are needed for a given time and place, other publications of the Weather Bureau should be consulted. P. R. M.